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# UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))

Attorney Docket No. 1995-033-12US

First Inventor or Application Identifier THATCHER ET AL.

Title METHODS AND COMPOSITIONS FOR MITIGATING PAIN

Express Mail Label No.

## APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: Assistant Commissioner for Patents  
Box Patent Application  
Washington, DC 20231

1. ☐ \* Fee Transmittal Form (e.g., PTO/SB/17)  
(Submit an original and a duplicate for fee processing)

2. ☒ Specification [Total Pages 92]  
(preferred arrangement set forth below)

- Descriptive title of the invention
- Cross References to Related Applications
- Statement Regarding Fed sponsored R & D
- Reference to Microfiche Appendix
- Background of the invention
- Brief Summary of the invention
- Brief Description of the Drawings (if filed)
- Detailed Description
- Claim(s)
- Abstract of the Disclosure

3. ☒ Drawing(s) (35 U.S.C. 113) [Total Sheets 15]

4. Oath or Declaration unsigned [Total Pages 3]

a. ☐ Newly executed (original or copy)

b. ☐ Copy from a prior application (37 C.F.R. § 1.63(d))  
(for continuation/divisional with Box 17 completed)  
[Note Box 5 below]

i. ☐ DELETION OF INVENTOR(S)  
Signed statement attached deleting  
inventor(s) named in the prior application,  
see 37 C.F.R. §§ 1.63(d)(2) and 1.33(b).

5. ☐ Incorporation By Reference (useable if Box 4b is checked)  
The entire disclosure of the prior application, from which a  
copy of the oath or declaration is supplied under Box 4b, is  
considered to be part of the disclosure of the accompanying  
application and is hereby incorporated by reference therein.

6. ☐ Microfiche Computer Program (Appendix)

7. Nucleotide and/or Amino Acid Sequence Submission  
(if applicable, all necessary)

a. ☐ Computer Readable Copy

b. ☐ Paper Copy (identical to computer copy)

c. ☐ Statement verifying identity of above copies

## ACCOMPANYING APPLICATION PARTS

8. ☐ Assignment Papers (cover sheet & document(s))

9. ☐ 37 C.F.R. § 3.73(b) Statement ☐ Power of Attorney  
(when there is an assignee)

10. ☐ English Translation Document (if applicable)

11. ☐ Information Disclosure Statement (IDS)/PTO-1449 ☐ Copies of IDS  
Citations

12. ☐ Preliminary Amendment

13. ☒ Return Receipt Postcard (MPEP 503)  
(Should be specifically itemized)

\* Small Entity Statement(s) ☐ Statement filed in prior application,  
(PTO/SB/09-12) Status still proper and desired

15. ☐ Certified Copy of Priority Document(s)  
(if foreign priority is claimed)

16. ☐ Other: .....

\* NOTE FOR ITEMS 1 & 14: IN ORDER TO BE ENTITLED TO PAY SMALL ENTITY  
FEES, A SMALL ENTITY STATEMENT IS REQUIRED (37 C.F.R. § 1.27), EXCEPT  
IF ONE FILED IN A PRIOR APPLICATION IS RELIED UPON (37 C.F.R. § 1.28).

17. If a CONTINUING APPLICATION, check appropriate box, and supply the requisite information below and in a preliminary amendment:

☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP)

of prior application No: \_\_\_\_\_/\_\_\_\_\_

Prior application information: Examiner \_\_\_\_\_

Group / Art Unit: \_\_\_\_\_

## 18. CORRESPONDENCE ADDRESS

☐ Customer Number or Bar Code Label

(Insert Customer No. or Attach bar code label here)

or ☐ Correspondence address below

Name

Stephen J. Scribner, Reg. No. 44,452

Address

PARTEQ INNOVATIONS

QUEEN'S UNIVERSITY

Biosciences Complex, Room 1625

City

Kingston, Ontario

State

Canada

Zip Code

K7L 3N6

Country

Canada

Telephone

(613) 533-2342

Fax

(613) 533-6853

Name (Print/Type)

Ralph A. Dowell

Registration No. (Attorney/Agent)

26,868

Signature

*Ralph A. Dowell*

Date

12/29/99

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**UNITED STATES OF AMERICA**

TO ALL WHOM IT MAY CONCERN:

BE IT KNOWN THAT WE:

Gregory R. J. THATCHER, 371 Alfred Street, Kingston, Ontario, K7K 4H6, Canada, citizen of United Kingdom,

and

Brian M. BENNETT, 36 Fairway Hill Crescent, Kingston, Ontario, K7M 2B4, Canada, citizen of Canada,

and

James N. REYNOLDS, 52 Herchmer Crescent, Kingston, Ontario, K7M 2V9, Canada, citizen of Canada,

and

Khem JHAMANDAS, 17 Jorene Drive, Kingston, Ontario, K7M 3X5, Canada, citizen of Canada

have invented:

**METHOD OF MITIGATING PAIN**

of which the following is a specification:

## METHODS AND COMPOSITIONS FOR MITIGATING PAIN

### FIELD OF THE INVENTION

This invention relates to nitrate esters and use thereof in mitigating pain and effecting analgesia. More particularly this invention relates to organic nitrates which have therapeutic utility as analgesics, anti-inflammatory agents and sedatives.

### BACKGROUND OF THE INVENTION

The nitrate ester, glyceryl trinitrate (GTN), or nitroglycerin, has been used as a vasodilator in the treatment of angina pectoris for over a hundred years, and the dominant, contemporary belief is that GTN exerts its therapeutic effect through *in vivo* release of nitric oxide (NO). Other organic nitrates (nitrate esters), such as isosorbide dinitrate, have also been identified as effective and clinically important vasodilators. NO itself has been identified as Endothelium Derived Relaxing Factor (EDRF) and several classes of compounds, for example nitrosothiols, in addition to organic nitrates, have been proposed as NO donors or NO prodrugs.

Several organic nitrates, in which an alkyl mononitrate is appended to a moiety with analgesic properties, such as aspirin (ASA) or a Non-Steroidal Anti-Inflammatory Drug (NSAID) have been reported as analgesics which possess reduced gastro-intestinal irritation and ulceration properties, purportedly through release of NO. The combination of the vasodilator nitroglycerin with opioid analgesics such as morphine, has been suggested to be effective in the management of both surgical and cancer pain. However, no attempt has been made to develop organic nitrates themselves as analgesic agents, that is, organic nitrates that do not rely on an ASA or NSAID moiety, nor an opiate, for analgesic properties. Thus, there is a need for synthetic organic nitrates as new and useful therapeutic agents for treatment and mitigation of pain associated with disease states and chemotherapy of those disease states.

### OBJECT OF THE INVENTION

It is an object of the present invention to provide methods and compositions for use in treating pain and/or conditions associated with pain. Another object of the present invention is to provide methods and compositions for providing analgesia and/or sedation.

## 5 BRIEF DESCRIPTION OF THE INVENTION

The present invention is based, at least in part, on the recognition that, although the potent vasodilatory effects of organic nitrates may be either (a) deleterious to or, alternatively, (b) synergistic with their analgesic effects, *regulation* of these two effects is required for the development of therapeutic agents useful in treatment and mitigation of pain.

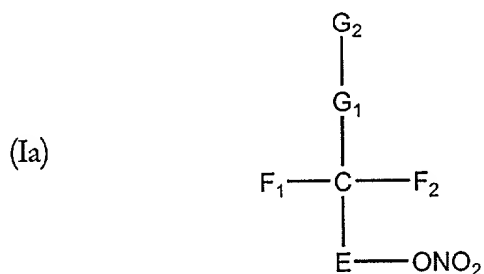
10 Pain may be treated or mitigated by, for example, an analgesic, anti-inflammatory and/or sedative agent.

Possible deleterious effects of organic nitrates may arise, for example, through an NO-donor potentiating hyperalgesia via a cyclic guanosine-3,5-monophosphate (cGMP)-independent mechanism. Alternatively, synergistic effects of organic nitrates may arise, for  
15 example, through the ability of an NO-donor to induce analgesia by activation of soluble guanylyl cyclase (GCase) and elevation of cGMP levels. The present invention relates to methods for treating or mitigating pain through use of an organic nitrate, wherein regulation of these two effects is achieved. According to the invention, selection of an appropriate organic nitrate provides modulation and balance between the ability of the organic nitrate to  
20 release NO and its potency for GCase activation. Inasmuch as gastrointestinal toxicity is known to be a deleterious side effect of some analgesic drugs and that NO donor molecules are gastro-protective, it is set forth herein that therapeutic analgesia can be achieved through utilization of an appropriate organic nitrate. This statement is based, at least in part, on bioassay data on such compounds.

25 This invention provides methods and compositions which are useful in treating pain, inhibiting inflammation, and/or providing analgesia. Methods of the invention involve administering to a subject a therapeutic compound (nitrate ester) which provides analgesia. The methods and compositions of the invention are useful for the treatment and mitigation of pain associated with disorders and disease states and chemotherapy of those disease states. The

methods and compositions of the invention can be used therapeutically to treat acute, chronic and/or inflammatory pain in conditions such as, but not limited to, nerve injury, post-herpetic neuralgia, arthritis, diabetic neuropathy, dysmenorrhea, endometriosis, phantom limb pain, pain associated with cancer and post-operative pain, or can be used prophylactically in a subject susceptible or predisposed to these conditions. In certain preferred embodiments, a therapeutic compound used in the method of the invention interacts with guanylyl cyclase, effecting analgesia. In other preferred embodiments, a therapeutic compound used in the method of the invention modulates levels of the cyclic nucleotides cyclic guanosine-3',5'-monophosphate (cGMP) and cyclic adenosine-3',5'- monophosphate (cAMP).

In one aspect, the invention provides a method for treating pain, treating or inhibiting inflammation, providing analgesia, providing sedation, mitigating anxiety and/or providing anaesthesia in a subject, comprising administering to a subject in need thereof an effective amount of a therapeutic compound, wherein the therapeutic compound is of the formula (Ia):



in which E, F<sub>1</sub>, F<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub> are the same or different organic radicals which may be joined in cyclic ring systems, and which may contain inorganic counterions;

with the proviso that when E and G<sub>1</sub> are methylene groups and F<sub>1</sub> is H, G<sub>2</sub> is not a nitrate group, nor R<sup>N</sup>-Z<sup>N</sup>-;

wherein R<sup>N</sup> is any aryl or heteroaryl group and Z<sup>N</sup> is (CO)<sub>mm</sub>-X<sup>N</sup><sub>nn</sub>-Y<sup>N</sup><sub>oo</sub>;

wherein mm, nn, oo are 0 or 1 and X<sup>N</sup>, Y<sup>N</sup> are NH, NR<sup>NN</sup>, O or CH<sub>2</sub>;

wherein R<sup>NN</sup> is a short chain alkyl group (C<sub>1</sub> - C<sub>12</sub>).

In a preferred embodiment,  $F_2$  is a nitrate group and  $E$ ,  $F_1$ ,  $G_1$ ,  $G_2$  are the same or different organic radicals which may be joined in cyclic ring systems, and which may contain inorganic counterions;

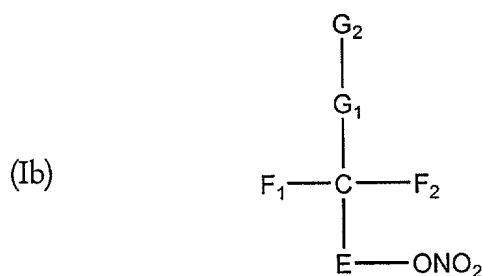
with the proviso that when  $E$  and  $G_1$  are methylene groups and  $F_1$  is  $H$ ,  $G_2$  is not a nitrate group, nor  $R^N-Z^N$ ;

wherein  $R^N$  is any aryl or heteroaryl group and  $Z^N$  is  $(CO)_{mm}-X_{nn}^N-Y_{oo}^N$ ;

wherein  $mm$ ,  $nn$ ,  $oo$  are 0 or 1 and  $X^N, Y^N$  are  $NH$ ,  $NR^{NN}$ ,  $O$  or  $CH_2$ ;

wherein  $R^{NN}$  is a short chain alkyl group ( $C_1 - C_{12}$ )

In another aspect, the invention provides a method for treating pain, treating or inhibiting inflammation, providing analgesia, providing sedation, mitigating anxiety and/or providing anaesthesia in a subject, comprising administering to a subject in need thereof an effective amount of a therapeutic compound, wherein the therapeutic compound is of the formula (Ib):



in which  $F_2$  is an organic radical which may be joined in a cyclic ring system with  $G_2$ , and which may contain inorganic counterions;  $E$  and  $G_1$  are both methylene groups;  $F_1$  is  $H$ ; and  $G_2$  is  $R^N-Z^N$ ;

wherein  $R^N$  is an organic radical possessing a heteroaryl group containing  $P$  or  $S$  atoms where said  $P$  or  $S$  are positioned  $\beta$ ,  $\gamma$ , or  $\delta$  to a nitrate group as identified in formula I; and  $Z^N$  is  $W_{mm}^N-X_{nn}^N-Y_{oo}^N$ ;

wherein  $mm$ ,  $nn$  and  $oo$  are 0 or 1; and  $W^N, X^N, Y^N$  are  $NH$ ,  $NR^{NN}$ ,  $CO$ ,  $O$  or  $CH_2$ ;

wherein  $R^{NN}$  is a short chain alkyl group ( $C_1 - C_{12}$ ).

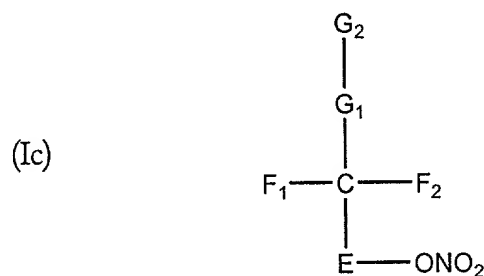
In a preferred embodiment,  $F_2$  is a nitrate group;  $E$  and  $G_1$  are methylene groups;  $F_1$  is  $H$ ; and  $G_2$  is  $R^N-Z^N$ ;

wherein  $R^N$  is an organic radical possessing an heteroaryl group containing P or S atoms where said P or S are positioned  $\beta$ ,  $\gamma$ , or  $\delta$  to a nitrate group as identified in formula I; and  $Z^N$  is  $W_{mm}^N-X_{nn}^N-Y_{oo}^N$ ;

wherein mm, nn, oo are 0 or 1 and  $W^N, X^N, Y^N$  are  $NH, NR^{NN}, CO, O$  or  $CH_2$ ;

wherein  $R^{NN}$  is a short chain alkyl group ( $C_1 - C_{12}$ ).

In another aspect, the invention provides a method for treating pain, treating or inhibiting inflammation, providing analgesia, providing sedation, mitigating anxiety and/or providing anaesthesia in a subject, comprising administering to a subject in need thereof an effective amount of a therapeutic compound, wherein the therapeutic compound is of the formula (Ic):



in which E is  $(R^1R^2C)_m$  and  $G_2-G_1-CF_1F_2-$  is  $R^{19}-(R^3R^4C)_p-(R^{17}R^{18}C)_n-$ ;

wherein: m, n, p are integers from 0 to 10;

$R^{3,17}$  are each independently hydrogen, a nitrate group, or A; and

$R^{1,4}$  are each independently hydrogen, or A;

where A is selected from a substituted or unsubstituted aliphatic group (preferably a branched or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain, which optionally may contain O, S,  $NR^6$  and unsaturations in the chain, optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; an unsubstituted or substituted cyclic aliphatic moiety having from 3 to 7 carbon atoms in the aliphatic ring, which optionally may contain O, S,  $NR^6$  and unsaturations in the ring, optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; an unsubstituted or substituted aliphatic moiety constituting a linkage of from 0 to 5 carbons, between  $R^1$  and  $R^3$

and/or between  $R^{17}$  and  $R^4$ , which optionally may contain O, S,  $NR^6$  and unsaturations in the linkage, and optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups); a substituted or unsubstituted aliphatic group (preferably a branched, cyclic or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain) containing  
 5 carbonyl linkages (e.g.,  $C=O$ ,  $C=S$ ,  $C=NOH$ ), which optionally may contain O, S,  $NR^6$  and unsaturations in the chain, optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; a substituted or unsubstituted aryl group; a heterocyclic group; amino (including alkylamino, dialkylamino (including cyclic amino, diamino and triamino moieties), arylamino, diarylamino, and alkylarylamino); hydroxy; alkoxy; a substituted or unsubstituted  
 10 aryloxy;

wherein X is F, Br, Cl,  $NO_2$ ,  $CH_2$ ,  $CF_2$ , O, NH, NMe, CN, NHOH,  $N_2H_3$ ,  $N_2H_2R^{13}$ ,  $N_2HR^{13}R^{14}$ ,  $N_3$ , S, SCN,  $SCN_2H_2(R^{15})_2$ ,  $SCN_2H_3(R^{15})$ ,  $SC(O)N(R^{15})_2$ ,  $SC(O)NHR^{15}$ ,  $SO_3M$ , SH,  $SR^7$ ,  $SO_2M$ ,  $S(O)R^8$ ,  $S(O)_2R^9$ ,  $S(O)OR^8$ ,  $S(O)_2OR^9$ ,  $PO_2HM$ ,  $PO_3HM$ ,  $PO_3M_2$ ,  $P(O)(OR^{15})(OR^{16})$ ,  $P(O)(OR^{16})(OM)$ ,  $P(O)(R^{15})(OR^8)$ ,  $P(O)(OM)R^{15}$ ,  $CO_2M$ ,  $CO_2H$ ,  $CO_2R^{11}$ ,  
 15  $C(O)$ ,  $C(O)R^{12}$ ,  $C(O)(OR^{13})$ ,  $PO_2H$ ,  $PO_2M$ ,  $P(O)(OR^{14})$ ,  $P(O)(R^{13})$ , SO,  $SO_2$ ,  $C(O)(SR^{13})$ ,  $SR^5$ ,  $SSR^7$  or  $SSR^5$ ;

Y is F, Br, Cl,  $CH_3$ ,  $CF_2H$ ,  $CF_3$ , OH,  $NH_2$ ,  $NHR^6$ ,  $NR^6R^7$ , CN, NHOH,  $N_2H_3$ ,  $N_2H_2R^{13}$ ,  $N_2HR^{13}R^{14}$ ,  $N_3$ , S, SCN,  $SCN_2H_2(R^{15})_2$ ,  $SCN_2H_3(R^{15})$ ,  $SC(O)N(R^{15})_2$ ,  $SC(O)NHR^{15}$ ,  $SO_3M$ , SH,  $SR^7$ ,  $SO_2M$ ,  $S(O)R^8$ ,  $S(O)_2R^9$ ,  $S(O)OR^8$ ,  $S(O)_2OR^9$ ,  $PO_2HM$ ,  $PO_3M_2$ ,  
 20  $P(O)(OR^{15})(OR^{16})$ ,  $P(O)(OR^{16})(OM)$ ,  $P(O)(R^{15})(OR^8)$ ,  $P(O)(OM)R^{15}$ ,  $CO_2M$ ,  $CO_2H$ ,  $CO_2R^{11}$ ,  $C(O)R^{12}$ ,  $C(O)(OR^{13})$ ,  $C(O)(SR^{13})$ ,  $SR^5$ ,  $SSR^7$  or  $SSR^5$ , or does not exist;

$R^2$ ,  $R^5$ ,  $R^{18}$ ,  $R^{19}$  are optionally hydrogen, A or X-Y;

$R^6$ ,  $R^7$ ,  $R^8$ ,  $R^9$ ,  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ ,  $R^{14}$ ,  $R^{15}$ ,  $R^{16}$  are the same or different alkyl or acyl groups containing 1-24 carbon atoms which may contain 1-4  $ONO_2$  substituents; or  $C_1 - C_6$

25 connections to  $R^1 - R^4$  in cyclic derivatives which may contain 1-4  $ONO_2$  substituents; or are each independently hydrogen a nitrate group or A;

M is H,  $Na^+$ ,  $K^+$ ,  $NH_4^+$ ,  $N^+H_kR^{11}_{(4-k)}$  where k is 0-3; or other pharmaceutically acceptable counterion;



and with the proviso that when  $m = n = p = 1$  and  $R^{19}, R^2, R^{18}, R^1 = H$  and  $R^{17}, R^3$  are nitrate groups,  $R^4$  is not H.

In a preferred embodiment,  $R^{19}$  is X-Y.

In other embodiments,  $R^1$  and  $R^3$  are the same or different and selected from H and  $C_1$ -  
5  $C_4$  alkyl chains, which chains may include one O linking  $R^1$  and  $R^3$  to form pentosyl, hexosyl, cyclopentyl, or cyclohexyl rings, which rings may optionally bear hydroxyl substituents;

$R^2$  and  $R^4$  are the same or different and selected from H, a nitrate group,  $C_1$ - $C_4$  alkyl chains optionally bearing 1-3 nitrate groups, and acyl groups  $(-C(O)R^5)$ ;

$R^7, R^{11}$  are the same or different  $C_1 - C_8$  alkyl or acyl;

10  $R^5, R^6, R^8, R^9, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}$  are the same or different and are alkyl groups containing 1-12 carbon atoms which may contain 1-4  $ONO_2$  substituents; or  $C_1$  or  $C_2$  connections to  $R^1 - R^3$  in cyclic derivatives; and

M is H,  $Na^+$ ,  $K^+$ ,  $NH_4^+$  or  $N^+H_kR^{11}_{(4-k)}$ , where k is 0-3.

In other embodiments,  $m = 1, n = 0, p = 1$ .

15 In further embodiments, X is  $CH_2, O, NH, NMe, CN, NHOH, N_2H_3, N_2H_2R^{13}, N_2HR^{13}R^{14}, N_3, S, SCN, SCN_2H_2(R^{15})_2, SCN_2H_3(R^{15}), SC(O)N(R^{15})_2, SC(O)NHR^{15}, SO_3M, SH, SR^7, SO_2M, S(O)R^8, S(O)_2R^9, S(O)OR^8, S(O)_2OR^9, PO_3HM, PO_3M_2, P(O)(OR^{15})(OR^{16}), P(O)(OR^{16})(OM), P(O)(R^{15})(OR^8), P(O)(OM)R^{15}, CO_2M, CO_2H, CO_2R^{11}, C(O), C(O)R^{12}, C(O)(OR^{13}), PO_2M, P(O)(OR^{14}), P(O)(R^{13}), SO, SO_2, C(O)(SR^{13}),$  or  $SSR^4$ ; and

20 Y is  $CN, N_2H_2R^{13}, N_2HR^{13}R^{14}, N_3, SCN, SCN_2H_2(R^{15})_2, SC(O)N(R^{15})_2, SC(O)NHR^{15}, SO_3M, SR^4, SO_2M, PO_3HM, PO_3M_2, P(O)(OR^{15})(OR^{16}), P(O)(OR^{16})(OM), P(O)(R^{15})(OR^8), P(O)(OM)R^{15}, CO_2M, CO_2H, CO_2R^{11}, C(O)R^{12}, C(O)(SR^{13}), SR^5,$  or  $SSR^5$ , or does not exist.

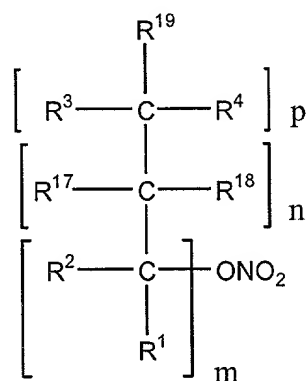
In yet further embodiments,  $R^5, R^6, R^8, R^9, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}$  are the same or different and are alkyls containing 1-12 carbon atoms; or  $C_1$  or  $C_2$  connections to  $R^1$  or  $R^3$  in  
25 cyclic derivatives;

X is  $CH_2, O, NH, NMe, S, SO_3M, SH, SR^7, SO_2M, S(O)R^8, S(O)_2R^9, S(O)OR^8, S(O)_2OR^9, PO_3M_2, P(O)(OR^{15})(OR^{16}), P(O)(OR^{16})(OM), P(O)(R^{15})(OR^8), PO_3HM$  or  $P(O)(OM)R^{15}$ ; and

Y is  $\text{SO}_2\text{M}$ ,  $\text{SO}_3\text{M}$ ,  $\text{PO}_3\text{HM}$ ,  $\text{PO}_3\text{M}_2$ ,  $\text{P}(\text{O})(\text{OR}^{15})(\text{OR}^{16})$ ,  $\text{P}(\text{O})(\text{OR}^{16})(\text{OM})$ ,  $\text{SR}^5$ ,  $\text{SR}^4$  or  $\text{SSR}^5$ , or does not exist.

In preferred embodiments, therapeutic compounds of the invention act as analgesic, sedative and/or anti-inflammatory agents. Preferred therapeutic compounds for use in the invention include compounds having the formula (Formula II):

(Formula II)



in which: m and n and p are integers from 0 to 10;

$\text{R}^{3,17}$  are each independently hydrogen; a nitrate group; or A;

$\text{R}^{1,4}$  are each independently hydrogen; or A;

where A is selected from: a substituted or unsubstituted aliphatic group (preferably a branched, or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain, which optionally may contain O, S,  $\text{NR}^6$  and unsaturations in the chain, optionally bearing from 1 to 4 hydroxy, or nitrate, or amino or aryl, or heterocyclic groups; an unsubstituted or substituted cyclic aliphatic moiety having from 3 to 7 carbon atoms in the aliphatic ring, which optionally may contain O, S,  $\text{NR}^6$  and unsaturations in the ring, optionally bearing from 1 to 4 hydroxy, or nitrate, or amino or aryl, or heterocyclic groups; an unsubstituted or substituted aliphatic moiety constituting a linkage of from 0 to 5 carbons, between  $\text{R}^1$  and  $\text{R}^3$  and/or between  $\text{R}^{17}$  and  $\text{R}^4$ , which optionally may contain O, S,  $\text{NR}^6$  and unsaturations in the linkage, and optionally bearing from 1 to 4 hydroxy, or nitrate, or amino or aryl, or heterocyclic groups); a substituted or unsubstituted aliphatic group (preferably a branched,

cyclic or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain), containing carbonyl linkages (e.g., C=O, C=S, C=NOH), which optionally may contain O, S, NR<sup>6</sup> and unsaturations in the chain, optionally bearing from 1 to 4 hydroxy, or nitrate, or amino or aryl, or heterocyclic groups; a substituted or unsubstituted aryl group; a heterocyclic group; amino (including alkylamino, dialkylamino (including cyclic amino, diamino and triamino moieties), arylamino, diarylamino, and alkylarylamino); hydroxy; alkoxy; a substituted or unsubstituted aryloxy.

R<sup>2</sup>, R<sup>5</sup>, R<sup>18</sup>, R<sup>19</sup> are optionally hydrogen; or A; or X-Y.

where X is F, Br, Cl, NO<sub>2</sub>, CH<sub>2</sub>, CF<sub>2</sub>, O, NH, NMe, CN, NHOH, N<sub>2</sub>H<sub>3</sub>, N<sub>2</sub>H<sub>2</sub>R<sup>13</sup>, N<sub>2</sub>HR<sup>13</sup>R<sup>14</sup>, N<sub>3</sub>, S, SCN, SCN<sub>2</sub>H<sub>2</sub>(R<sup>15</sup>)<sub>2</sub>, SCN<sub>2</sub>H<sub>3</sub>(R<sup>15</sup>), SC(O)N(R<sup>15</sup>)<sub>2</sub>, SC(O)NHR<sup>15</sup>, SO<sub>3</sub>M, SH, SR<sup>7</sup>, SO<sub>2</sub>M, S(O)R<sup>8</sup>, S(O)<sub>2</sub>R<sup>9</sup>, S(O)OR<sup>8</sup>, S(O)<sub>2</sub>OR<sup>9</sup>, PO<sub>2</sub>HM, PO<sub>3</sub>HM, PO<sub>3</sub>M<sub>2</sub>, P(O)(OR<sup>15</sup>)(OR<sup>16</sup>), P(O)(OR<sup>16</sup>)(OM), P(O)(R<sup>15</sup>)(OR<sup>8</sup>), P(O)(OM)R<sup>15</sup>, CO<sub>2</sub>M, CO<sub>2</sub>H, CO<sub>2</sub>R<sup>11</sup>, C(O), C(O)R<sup>12</sup>, C(O)(OR<sup>13</sup>), PO<sub>2</sub>H, PO<sub>2</sub>M, P(O)(OR<sup>14</sup>), P(O)(R<sup>13</sup>), SO, SO<sub>2</sub>, C(O)(SR<sup>13</sup>), SR<sup>5</sup>, SSR<sup>7</sup> or SSR<sup>5</sup>,

Y is F, Br, Cl, CH<sub>3</sub>, CF<sub>2</sub>H, CF<sub>3</sub>, OH, NH<sub>2</sub>, NHR<sup>6</sup>, NR<sup>6</sup>R<sup>7</sup>, CN, NHOH, N<sub>2</sub>H<sub>3</sub>, N<sub>2</sub>H<sub>2</sub>R<sup>13</sup>, N<sub>2</sub>HR<sup>13</sup>R<sup>14</sup>, N<sub>3</sub>, S, SCN, SCN<sub>2</sub>H<sub>2</sub>(R<sup>15</sup>)<sub>2</sub>, SCN<sub>2</sub>H<sub>3</sub>(R<sup>15</sup>), SC(O)N(R<sup>15</sup>)<sub>2</sub>, SC(O)NHR<sup>15</sup>, SO<sub>3</sub>M, SH, SR<sup>7</sup>, SO<sub>2</sub>M, S(O)R<sup>8</sup>, S(O)<sub>2</sub>R<sup>9</sup>, S(O)OR<sup>8</sup>, S(O)<sub>2</sub>OR<sup>9</sup>, PO<sub>2</sub>HM, PO<sub>3</sub>M<sub>2</sub>, P(O)(OR<sup>15</sup>)(OR<sup>16</sup>), P(O)(OR<sup>16</sup>)(OM), P(O)(R<sup>15</sup>)(OR<sup>8</sup>), P(O)(OM)R<sup>15</sup>, CO<sub>2</sub>M, CO<sub>2</sub>H, CO<sub>2</sub>R<sup>11</sup>, C(O)R<sup>12</sup>, C(O)(OR<sup>13</sup>), C(O)(SR<sup>13</sup>), SR<sup>5</sup>, SSR<sup>7</sup> or SSR<sup>5</sup>, or does not exist;

R<sup>6</sup>, R<sup>7</sup>, R<sup>8</sup>, R<sup>9</sup>, R<sup>10</sup>, R<sup>11</sup>, R<sup>12</sup>, R<sup>13</sup>, R<sup>14</sup>, R<sup>15</sup>, R<sup>16</sup> are the same or different alkyl or acyl groups containing 1-24 carbon atoms which may contain 1-4 ONO<sub>2</sub> substituents; or C<sub>1</sub> - C<sub>6</sub> connections to R<sup>1</sup> - R<sup>4</sup> in cyclic derivatives; or are each independently hydrogen; a nitrate group; or W.

M is H, Na<sup>+</sup>, K<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, N<sup>+</sup>H<sub>k</sub>R<sup>11</sup><sub>(4-k)</sub> where k is 0-3, or other pharmaceutically acceptable counterion;

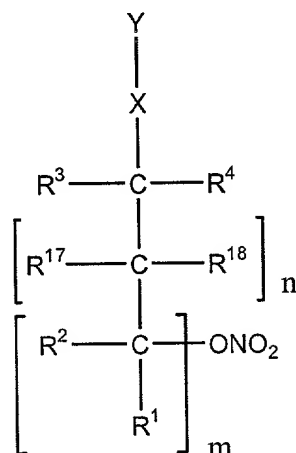
and with the proviso that, when m = n = p = 1; R<sup>19</sup>, R<sup>2</sup>, R<sup>18</sup>, R<sup>1</sup> = H; R<sup>17</sup>, R<sup>3</sup> are nitrate groups; that R<sup>4</sup> is not H or C<sub>1</sub> - C<sub>3</sub> alkyl.

In certain preferred embodiments, therapeutic compounds of the invention are analgesic, sedative and/or anti-inflammatory agents. Preferred therapeutic compounds for use

in the invention include compounds in which  $R^{19}$  is X-Y. In a particularly preferred embodiment:  $R^{19}$  is X-Y and  $R^5, R^6, R^8, R^9, R^{10}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}$  are the same or different alkyl groups containing 1-24 carbon atoms which may contain 1-4  $ONO_2$  substituents, or  $C_1$  or  $C_2$  connections to  $R^1 - R^3$  in cyclic derivatives;  $R^1$  and  $R^3$  are the same or different and selected from H,  $C_1$ - $C_4$  alkyl chains, which may include one O, linking  $R^1$  and  $R^3$  to form pentosyl, hexosyl, cyclopentyl, or cyclohexyl rings, which rings may optionally bear hydroxyl substituents;  $R^2$  and  $R^4$  are the same or different and selected from H, a nitrate group,  $C_1$ - $C_4$  alkyl optionally bearing 1-3 nitrate groups, and acyl groups ( $-C(O)R^5$ );  $R^7, R^{11}$  are the same or different  $C_1 - C_8$  alkyl or acyl.

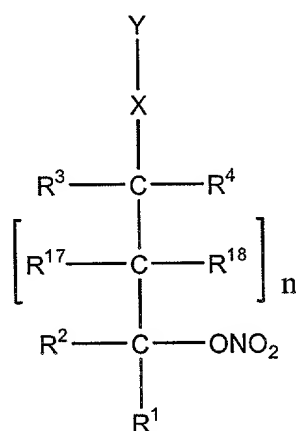
In certain embodiments in which  $R_{19}$  is X-Y,  $m, p = 1$ , and  $n = 0$ . In other embodiments in which  $R_{19}$  is X-Y, X is selected from  $CH_2, O, NH, NMe, CN, NHOH, N_2H_3, N_2H_2R^{13}, N_2HR^{13}R^{14}, N_3, S, SCN, SCN_2H_2(R^{15})_2, SCN_2H_3(R^{15}), SC(O)N(R^{15})_2, SC(O)NHR^{15}, SO_3M, SH, SR^7, SO_2M, S(O)R^8, S(O)_2R^9, S(O)OR^8, S(O)_2OR^9, PO_3HM, PO_3M_2, P(O)(OR^{15})(OR^{16}), P(O)(OR^{16})(OM), P(O)(R^{15})(OR^8), P(O)(OM)R^{15}, CO_2M, CO_2H, CO_2R^{11}, C(O), C(O)R^{12}, C(O)(OR^{13}), PO_2M, P(O)(OR^{14}), P(O)(R^{13}), SO, SO_2, C(O)(SR^{13}), SSR^4$ . In another embodiment in which  $R_{19}$  is X-Y, Y is selected from  $CN, N_2H_2R^{13}, N_2HR^{13}R^{14}, N_3, SCN, SCN_2H_2(R^{15})_2, SC(O)N(R^{15})_2, SC(O)NHR^{15}, SO_3M, SR^4, SO_2M, PO_3HM, PO_3M_2, P(O)(OR^{15})(OR^{16}), P(O)(OR^{16})(OM), P(O)(R^{15})(OR^8), P(O)(OM)R^{15}, CO_2M, CO_2H, CO_2R^{11}, C(O)R^{12}, C(O)(SR^{13}), SR^5, SSR^5$ , or does not exist. In a further embodiment, X and/or Y contain a sulfur-containing functional group. In certain preferred embodiments, the compound of the invention comprises a heterocyclic functionality, more preferably, a nucleoside or nucleobase. In further preferred embodiments, the compound of the invention comprises a carbocyclic functionality, more preferably, a steroidal or carbohydrate moiety.

In another aspect, a therapeutic compound of the invention is represented by the formula (Formula III):



in which: m is 1-10;  $R^{1-18}$ , X, and Y have the meaning as defined above. In certain preferred embodiments,  $R^6 - R^{16}$  are the same or different alkyl or acyl groups containing 1-24 carbon atoms which may contain 1-4  $\text{ONO}_2$  substituents, or  $C_1 - C_6$  connections to  $R^1 - R^4$  in cyclic derivatives. In certain preferred embodiments,  $R^{18}$  is A and  $n = 1$ .

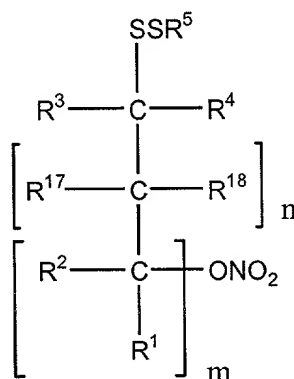
In preferred embodiments, a therapeutic compound of the invention is represented by the formula (Formula IV):



in which:  $R^3, R^1 = H$ ; n,  $R^2, R^{4-18}$ , X, and Y have the meaning as defined above. In preferred embodiments, X is  $\text{CH}_2$  or does not exist, and Y is selected from, F, Br, Cl,  $\text{CH}_3$ ,  $\text{CF}_2\text{H}$ ,  $\text{CF}_3$ , OH,  $\text{NH}_2$ ,  $\text{NHR}_6$ ,  $\text{NR}_6\text{R}_7$ , CN,  $\text{NHOH}$ ,  $\text{N}_2\text{H}_3$ ,  $\text{N}_2\text{H}_2\text{R}_{13}$ ,  $\text{N}_2\text{HR}_{13}\text{R}_{14}$ ,  $\text{N}_3$ , S,

SCN,  $\text{SCN}_2\text{H}_2(\text{R}_{15})_2$ ,  $\text{SCN}_2\text{H}_3(\text{R}_{15})$ ,  $\text{SC}(\text{O})\text{N}(\text{R}_{15})_2$ ,  $\text{SC}(\text{O})\text{NHR}_{15}$ ,  $\text{SO}_3\text{M}$ ,  $\text{SH}$ ,  $\text{SR}_7$ ,  $\text{SO}_2\text{M}$ ,  $\text{S}(\text{O})\text{R}_8$ ,  $\text{S}(\text{O})_2\text{R}_9$ ,  $\text{S}(\text{O})\text{OR}_8$ ,  $\text{S}(\text{O})_2\text{OR}_9$ ,  $\text{PO}_2\text{HM}$ ,  $\text{PO}_3\text{M}_2$ ,  $\text{P}(\text{O})(\text{OR}_{15})(\text{OR}_{16})$ ,  $\text{P}(\text{O})(\text{OR}_{16})(\text{OM})$ ,  $\text{P}(\text{O})(\text{R}_{15})(\text{OR}_8)$ ,  $\text{P}(\text{O})(\text{OM})\text{R}_{15}$ ,  $\text{CO}_2\text{M}$ ,  $\text{CO}_2\text{H}$ ,  $\text{CO}_2\text{R}_{11}$ ,  $\text{C}(\text{O})\text{R}_{12}$ ,  $\text{C}(\text{O})(\text{OR}_{13})$ ,  $\text{C}(\text{O})(\text{SR}_{13})$ ,  $\text{SR}_5$ ,  $\text{SSR}_7$  or  $\text{SSR}_5$ . In certain preferred embodiments,  $\text{R}_2$  and  $\text{R}_4$  are optionally H, a nitrate group or a connection to  $\text{R}_5\text{-R}_{16}$  in cyclic derivatives.

In certain preferred embodiments, a compound of the invention is represented by the formula (Formula V):



in which  $m$ ,  $n$ ,  $\text{R}^{1-18}$ ,  $\text{X}$ , and  $\text{Y}$  have the meaning as defined above.

In another aspect, the invention includes novel compounds useful for treating pain, mitigating inflammation, effecting analgesia and/or providing sedation. The compounds of the invention can be represented by the structures shown hereinbelow, for example, the structures of Formula III, IV and V. Novel compounds of the invention include nitrates IIIr - IIIaj, IVn - Ivt, and Vd - Vag, whose syntheses are described in the following examples.

The invention also provides methods for treating a disease state associated with inflammation, comprising administering to a subject an effective amount of a therapeutic compound having a formula set forth above, such that a disease state associated with inflammation is treated.

The invention further provides methods for treating a disease state or disorder in which a level of sedation is desired, comprising administering to a subject an effective amount of a sedative therapeutic compound having a formula set forth above, such that a disease state or disorder is treated.

The invention provides methods for effecting analgesia comprising administering to a subject an effective amount of a therapeutic compound having a formula set forth above, such that analgesia is effected.

The invention further provides novel pharmaceutical compositions for treating pain, mitigating inflammation, effecting analgesia and/or effecting sedation. A said pharmaceutical composition comprises a therapeutic compound of the invention in an effective amount for the particular indication and a pharmaceutically acceptable vehicle.

The invention also provides packaged pharmaceutical compositions for treating pain, mitigating inflammation, effecting analgesia and/or effecting sedation. The packaged pharmaceutical compositions include a therapeutic compound of the invention and instructions for using the pharmaceutical composition for treatment of inflammation and/or pain.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing the effect of IVd neat (diamonds); with added L-cysteine (2mM, triangles); with added dithiothreitol (2mM, DTT, squares); on soluble GCase activity in rat aorta homogenate normalized to the maximal GTN response. Bars represent the mean  $\pm$  standard errors calculated separately for each point.

Figure 2 is a graph showing the comparison of GTN (squares), IIIIm (circles) and IVh (triangles) with added L-cysteine (1 mM) on soluble GCase activity in rat aorta homogenate (a), and rat hippocampus homogenate (b). Data points represent the mean of duplicate determinations carried out in identical GCase preparations.

Figure 3 is a graph showing the comparison of GTN (squares), Va (circles) and Vb (triangles) with added L-cysteine(1 mM) on soluble GCase activity in rat aorta homogenate (a), and rat hippocampus homogenate (b). Data points represent the mean  $\pm$  standard errors calculated separately for each point (n=8-11).

Figure 4 is a graph showing the comparison of cyclic GMP accumulation in isolated rat aorta induced by diluent (basal, open bar), GTN (filled bar), Va (stippled bar), or IIIIm (hatched bar). Segments of rat aorta were exposed to diluent, 1  $\mu$ M drug (a), or 10 $\mu$ M drug (b)

for 1 min and cyclic GMP content determined by radioimmunoassay. Data are the mean  $\pm$  standard errors (a, n=8; b, n=5).

Figure 5 is a graph showing the comparison of cyclic GMP accumulation in isolated rat aorta induced by diluent (basal, open bar), GTN (filled bar), IVk (stippled bar), Vb (cross-hatched bar), or Vc (hatched bar). Segments of rat aorta were exposed to diluent, 1  $\mu$ M drug (a), or 10  $\mu$ M drug (b) for 1 min and cyclic GMP content determined by radioimmunoassay. Data are the mean  $\pm$  standard errors (a, n=5; b, n=4).

Figure 6 is a graph showing cyclic GMP accumulation in rat hippocampal slices induced by diluent (basal, open bar), GTN (filled bar), and Va (stippled bar). Sections of rat hippocampus (400  $\mu$ m) were prepared and exposed to diluent, 10  $\mu$ M drug (a) or 100  $\mu$ M drug (b) for 3 min and cyclic GMP content determined by radioimmunoassay. Data are the mean  $\pm$  standard errors (a, n=4; b, n=5).

Figure 7 is a graph showing the comparison of relaxation of isolated rat aorta induced by GTN (squares), Va (open triangles), compound IVc (diamonds), compound IVd (open squares), compound IVf (triangles), and compound IVg (open diamonds). Data points represent the mean  $\pm$  standard errors (n=5-8).

Figure 8 is a graph showing the comparison of relaxation of isolated rat aorta induced by GTN (squares), IVk (open triangles), Vb (diamonds), IIIIm (open squares), Vc (triangles), and IVh (open diamonds). Data points represent the mean  $\pm$  standard errors (n=3-8).

Figure 9 is a graph showing the comparison of the percent change in mean arterial pressure (MAP) in conscious unrestrained rats after subcutaneous administration of 400  $\mu$ mol/kg GTN (squares) or Va (open circles). Data points represent the mean  $\pm$  standard errors (n=6).

Figure 10 is a graph showing the comparison of the percent change in mean arterial pressure in Inactin anaesthetized rats after intravenous bolus injection of GTN (squares) or Va (open circles). Data points represent the mean  $\pm$  standard errors (n=4).

Figure 11 is a graph showing the plasma levels ( $\mu$ M) of Vb (circles) and its mononitrate metabolite Vc (open squares) after subcutaneous administration of 200  $\mu$ mol/kg Vb in conscious unrestrained rats. Data points represent the mean of two experiments.



Figure 12 is a graph showing the relaxation induced by compound IVd (a) and IVc (b) in untreated (squares) and GTN-tolerant (circles) isolated rat aorta. Aortae were made tolerant by treatment with 0.5 mM GTN for 30 min. Data points represent the mean  $\pm$  standard deviation ( $n = 3-6$ ).

Figure 13 is a graph showing the effect of Vm and Va in the mouse writhing test. GT Vm produced a dose-dependent analgesic effect (A). Va also produced analgesia in the mouse writhing test (B). Data are mean  $\pm$  standard errors ( $n = 10-20$ ).

Figure 14 is a graph showing the effect of Vm on paw flinches in rats after injection of formalin into the footpad. In comparison to vehicle-treated control animals, Vm decreased the initial pain response to formalin injection (at time = 0, \*,  $p < 0.05$ ), and the secondary hyperalgesia that developed between 20 and 40 minutes after formalin injection (A). For each animal, a cumulative score (total number of flinches over 60 minutes) was calculated (B). Vm significantly decreased cumulative paw flinches for 60 minutes after formalin injection. Data are mean  $\pm$  standard errors ( $N = 6-7$ ).

Figure 15 is a graph showing (A) the effects of chlormethiazole (CHLOR) and IVk (200  $\mu$ M of each) on the membrane current induced by 10  $\mu$ M GABA in a *Xenopus* oocyte expressing the  $\alpha 1\beta 1\gamma 2L$  isoform of human recombinant GABA<sub>A</sub> receptors, and (B) the effect of IVk on loss of the righting reflex in mice after intraperitoneal injection of 100 mg/kg and 200 mg/kg. Data in part B are mean  $\pm$  standard error for three animals at each dose.

## DETAILED DESCRIPTION OF THE INVENTION

In accordance with the invention there are provided methods and compositions useful in the treatment of pain. Methods of the invention involve administering to a subject an effective amount of a therapeutic compound which provides analgesia, mitigates inflammation and/or provides sedation. In some embodiments, the invention provides prophylactic methods for avoiding or pre-empting pain, inflammation and the like in a subject. For example, the subject may be susceptible or predisposed to these conditions, e.g., arthritic. Alternatively, the subject may be undergoing a course of treatment, e.g. cancer chemotherapy,

which produces pain, inflammation or the like as a side effect. Methods of the invention may be practiced prior to, concurrently with, or after such a course of treatment.

In accordance with the invention, analgesic, anti-inflammatory and/or sedative activity can be effected by modulating an interaction with guanylyl cyclase (GCase; the enzyme responsible for cGMP production in various areas of the body), and/or by modulating levels of cGMP and cAMP messenger molecules.

As used herein, the term "treating" pain encompasses preventing, ameliorating, mitigating and/or managing pain and/or conditions that may cause pain, such as inflammation. As used herein, "inhibiting" pain or inflammation encompasses preventing, reducing and halting progression of same. The terms "organic nitrate" and "nitrate ester" are used interchangeably herein, with no distinction drawn between them.

According to one aspect of the invention, there is provided a method for treating pain in a subject, comprising administering to the subject an effective amount of a compound (nitrate ester) which effects analgesia in the subject. Preferably, analgesia is effected by stimulating GCase. *In vivo*, GCase activation is effected by nitric oxide (NO), the proximal activator of GCase, which is generated endogenously by enzyme action on arginine in response to many biological triggers (J. R. Stone and M. A. Marletta, *Biochemistry* (1996) 35, 1093). One of the major targets for organic nitrates is GCase activation, resulting in the production of cGMP. In this respect, organic nitrates act as NO-surrogates. In some cases, there is evidence that organic nitrates may act also as NO-donors, but these two properties should be differentiated and can be modulated by choice of the appropriate organic nitrate.

Experimental evidence obtained in a number of *in vivo* model systems supports the notion that elevated levels of cGMP help effect analgesia. Sodium nitroprusside (SNP), which releases NO non-enzymatically, blocked the hyperalgesic effect of prostaglandin (PGE<sub>2</sub>) in a rat paw pressure test (Ferreira *et al.*, 1991). Moreover, this effect was potentiated by an inhibitor of cGMP phosphodiesterase, and blocked by an inhibitor of GCase (Ferreira *et al.*, 1991). The peripheral analgesic effects of morphine were attributed to elevations of cGMP levels in sensory nerve fibres (Ferreira *et al.*, 1991; Granados-Soto *et al.*, 1997) in both the rat paw pressure and formalin tests, since inhibition of GCase activity attenuated the analgesic

effects of locally applied morphine. Activation of the NO-cGMP system by NO donors such as SNP has also been reported to potentiate beta-endorphin-induced analgesia in thermal tail-flick test in mice (Xu *et al.*, 1995), an effect that was potentiated by a selective inhibitor (zaprinast) of a cGMP-specific phosphodiesterase.

5        Sensitization of sensory nerve fibres leading to hyperalgesia is assumed to involve increased concentrations of cAMP and calcium ions in sensory neurons, a process that may be attenuated or counteracted by activation of the NO-cGMP pathway (Ferreira, 1993; Cunha *et al.*, 1999).

10        In accordance with another embodiment of the invention, administration of an effective amount of a therapeutic compound to a subject effects analgesia in the subject by modulating levels of cAMP and/or cGMP. For example, it has been shown that NO-donors modulate hyperalgesia via modulation of levels of cAMP, separately from and in addition to modulation of cGMP levels, in rat models of pain and nociceptor sensitization (Aley *et al.* 1998). Thus, modulation of cAMP/cGMP levels is expected to be effective in inducing analgesia and in pain management in individuals suffering injury, disease or aging.

15        In a further embodiment of the invention, there is provided a method for treating or inhibiting inflammation in a subject, comprising administering to the subject an effective amount of a compound which mitigates inflammation in the subject. Preferably, inflammation is mitigated by modulation of levels of cGMP/cAMP. Inflammatory hyperalgesia has been shown to be linked directly to the NO-cGMP pathway (Ferreira, 1993).

20        We have shown in our co-pending application USSN 09/267,379, filed March 15, 1999, which is hereby incorporated by reference, that novel nitrate esters have differential effects to activate soluble GCase and to cause cGMP accumulation in vascular and brain tissue. Further, we have shown that the structure of the organic nitrate can be varied to alter potency and efficacy towards both activation of GCase and accumulation of cGMP and effects resulting from these processes in intact tissue, such as aortic strip relaxation. Activation of GCase and accumulation of cGMP have been shown to be important in the induction of analgesia. We show herein that novel organic nitrates are effective analgesics in animal models of pain management. The mouse writhing model, with a relatively short time course of minutes, is a

preclinical model of acute pain; whereas formalin injection in the rat paw is a preclinical model of acute and sensitization pain with a time course of minutes to hours, which effectively mimics the hyperalgesia/allodynia underlying pain due to tissue damage (Yaksh, 1999).

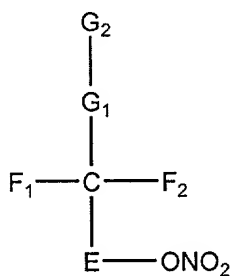
5 In a further embodiment, the invention relates to a method for providing sedation and/or anaesthesia in a subject, comprising administering to the subject an effective amount of a compound (organic nitrate) which effects sedation or anaesthesia in the subject. In certain aspects, the invention provides methods and compositions useful for reducing anxiety, and/or aiding or inducing sleep.

10  $\gamma$ -Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian (including human) central nervous system. GABA acts on three major classes of neurotransmitter receptor, designated type A (GABA<sub>A</sub>), type B (GABA<sub>B</sub>) and type C (GABA<sub>C</sub>). GABA<sub>A</sub> receptors play an important role in regulating many behavioural and physiological functions. Thus, drugs that modulate GABA<sub>A</sub> receptor function are among the  
15 most widely used in clinical medicine. For example, drugs that selectively potentiate GABA<sub>A</sub> receptor function (such as the benzodiazepines) are extensively used to relieve anxiety, produce sedation and induce sleep. Given the importance of this receptor mechanism in clinical medicine, there is a constant search for new chemical entities that modulate GABA<sub>A</sub> function, as the currently available drugs have several side effects, including ataxia, amnesia,  
20 tolerance and physical dependence. We show herein that organic nitrates that act as positive allosteric modulators of GABA<sub>A</sub> receptor function have sedative properties in the whole animal that are comparable to known drugs. This effect of organic nitrates has not previously been recognized or reported. Our findings provide direct evidence that nitrate esters are useful as sedative agents. Such agents are useful as therapeutics for treating conditions such as,  
25 for example, anxiety and pain associated with disease states; and as hypnotic agents. According to the invention, nitrate esters may also be employed prophylactically, to prevent or reduce anxiety, or to aid sleep.

Therapeutic compounds of the invention comprise at least one nitrate group. The nitrate group(s) can optionally be covalently bound to a carrier (e.g., an aromatic group, an

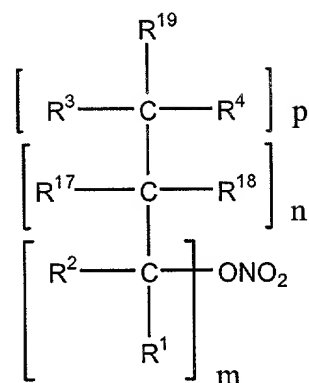
aliphatic group, peptide, steroid, nucleobase, nucleoside, peptidomimetic, steroidomimetic, or nucleoside analogue, or the like). In addition to functioning as a carrier for the nitrate functionality, the carrier molecule can enable the compound to traverse biological membranes and to be biodistributed preferentially, without excessive or premature metabolism. Further, in addition to functioning as a carrier for the nitrate functionality, the carrier molecule can enable the compound to exert amplified analgesic, sedative, or anti-inflammatory effects through synergism with the nitrate functionality.

In one embodiment, the invention provides a method comprising administering to a subject an effective amount of a therapeutic compound which has at least one nitrate group and is capable of effecting analgesia. In another embodiment, the therapeutic compound is capable of mitigating inflammation. In a further embodiment, the therapeutic compound is capable of effecting sedation. In the respective embodiments, the therapeutic compound has the formula (Formula I):



wherein: E, F<sub>1</sub>, F<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub> are the same or different organic radicals which may be joined in cyclic ring systems, and which may contain inorganic counterions.

In further aspects of the invention, therapeutic compounds of the invention effect analgesia, effect sedation and/or mitigate inflammation in a subject to which the therapeutic compound is administered, and have the formula (Formula II).



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in which: m, n, p are integers from 0 to 10;  $\text{R}^{3,17}$  are each independently hydrogen; a nitrate group; or A;  $\text{R}^{1,4}$  are each independently hydrogen; or A; where A is selected from: a substituted or unsubstituted aliphatic group (preferably a branched, or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain, which optionally may contain O, S,  $\text{NR}^6$  and unsaturations in the chain, optionally bearing from 1 to 4 hydroxy, or nitrate, or amino or aryl, or heterocyclic groups; an unsubstituted or substituted cyclic aliphatic moiety having from 3 to 7 carbon atoms in the aliphatic ring, which optionally may contain O, S,  $\text{NR}^6$  and unsaturations in the ring, optionally bearing from 1 to 4 hydroxy, or nitrate, or amino or aryl, or heterocyclic groups; an unsubstituted or substituted aliphatic moiety constituting a linkage of from 0 to 5 carbons, between  $\text{R}^1$  and  $\text{R}^3$  and/or between  $\text{R}^{17}$  and  $\text{R}^4$ , which optionally may contain O, S,  $\text{NR}^6$  and unsaturations in the linkage, and optionally bearing from 1 to 4 hydroxy, or nitrate, or amino or aryl, or heterocyclic groups); a substituted or unsubstituted aliphatic group (preferably a branched, cyclic or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain), containing carbonyl linkages (e.g.,  $\text{C}=\text{O}$ ,  $\text{C}=\text{S}$ ,  $\text{C}=\text{NOH}$ ), which optionally may contain O, S,  $\text{NR}^6$  and unsaturations in the chain, optionally bearing from 1 to 4 hydroxy, or nitrate, or amino or aryl, or heterocyclic groups; a substituted or unsubstituted aryl group; a heterocyclic group; amino (including alkylamino, dialkylamino (including cyclic amino, diamino and triamino moieties), arylamino, diarylamino, and alkylarylamino); hydroxy; alkoxy; a substituted or unsubstituted aryloxy;  $\text{R}^2$ ,  $\text{R}^5$ ,  $\text{R}^{18}$ ,  $\text{R}^{19}$  are optionally hydrogen; or A; or X-Y; where X is F, Br, Cl,  $\text{NO}_2$ ,  $\text{CH}_2$ ,  $\text{CF}_2$ , O, NH, NMe, CN, NHOH,  $\text{N}_2\text{H}_3$ ,  $\text{N}_2\text{H}_2\text{R}^{13}$ ,  $\text{N}_2\text{HR}^{13}\text{R}^{14}$ ,  $\text{N}_3$ , S, SCN,

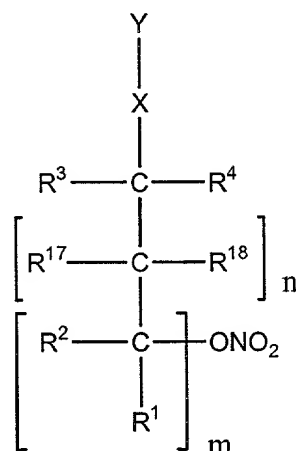
$\text{SCN}_2\text{H}_2(\text{R}^{15})_2$ ,  $\text{SCN}_2\text{H}_3(\text{R}^{15})$ ,  $\text{SC}(\text{O})\text{N}(\text{R}^{15})_2$ ,  $\text{SC}(\text{O})\text{NHR}^{15}$ ,  $\text{SO}_3\text{M}$ ,  $\text{SH}$ ,  $\text{SR}^7$ ,  $\text{SO}_2\text{M}$ ,  $\text{S}(\text{O})\text{R}^8$ ,  
 $\text{S}(\text{O})_2\text{R}^9$ ,  $\text{S}(\text{O})\text{OR}^8$ ,  $\text{S}(\text{O})_2\text{OR}^9$ ,  $\text{PO}_2\text{HM}$ ,  $\text{PO}_3\text{HM}$ ,  $\text{PO}_3\text{M}_2$ ,  $\text{P}(\text{O})(\text{OR}^{15})(\text{OR}^{16})$ ,  
 $\text{P}(\text{O})(\text{OR}^{16})(\text{OM})$ ,  $\text{P}(\text{O})(\text{R}^{15})(\text{OR}^8)$ ,  $\text{P}(\text{O})(\text{OM})\text{R}^{15}$ ,  $\text{CO}_2\text{M}$ ,  $\text{CO}_2\text{H}$ ,  $\text{CO}_2\text{R}^{11}$ ,  $\text{C}(\text{O})$ ,  $\text{C}(\text{O})\text{R}^{12}$ ,  
 $\text{C}(\text{O})(\text{OR}^{13})$ ,  $\text{PO}_2\text{H}$ ,  $\text{PO}_2\text{M}$ ,  $\text{P}(\text{O})(\text{OR}^{14})$ ,  $\text{P}(\text{O})(\text{R}^{13})$ ,  $\text{SO}$ ,  $\text{SO}_2$ ,  $\text{C}(\text{O})(\text{SR}^{13})$ ,  $\text{SR}^5$ ,  $\text{SSR}^7$  or  $\text{SSR}^5$ ;  
5 Y is F, Br, Cl,  $\text{CH}_3$ ,  $\text{CF}_2\text{H}$ ,  $\text{CF}_3$ , OH,  $\text{NH}_2$ ,  $\text{NHR}^6$ ,  $\text{NR}^6\text{R}^7$ , CN,  $\text{NHOH}$ ,  $\text{N}_2\text{H}_3$ ,  $\text{N}_2\text{H}_2\text{R}^{13}$ ,  
 $\text{N}_2\text{HR}^{13}\text{R}^{14}$ ,  $\text{N}_3$ , S, SCN,  $\text{SCN}_2\text{H}_2(\text{R}^{15})_2$ ,  $\text{SCN}_2\text{H}_3(\text{R}^{15})$ ,  $\text{SC}(\text{O})\text{N}(\text{R}^{15})_2$ ,  $\text{SC}(\text{O})\text{NHR}^{15}$ ,  $\text{SO}_3\text{M}$ ,  
 $\text{SH}$ ,  $\text{SR}^7$ ,  $\text{SO}_2\text{M}$ ,  $\text{S}(\text{O})\text{R}^8$ ,  $\text{S}(\text{O})_2\text{R}^9$ ,  $\text{S}(\text{O})\text{OR}^8$ ,  $\text{S}(\text{O})_2\text{OR}^9$ ,  $\text{PO}_2\text{HM}$ ,  $\text{PO}_3\text{M}_2$ ,  $\text{P}(\text{O})(\text{OR}^{15})(\text{OR}^{16})$ ,  
 $\text{P}(\text{O})(\text{OR}^{16})(\text{OM})$ ,  $\text{P}(\text{O})(\text{R}^{15})(\text{OR}^8)$ ,  $\text{P}(\text{O})(\text{OM})\text{R}^{15}$ ,  $\text{CO}_2\text{M}$ ,  $\text{CO}_2\text{H}$ ,  $\text{CO}_2\text{R}^{11}$ ,  $\text{C}(\text{O})\text{R}^{12}$ ,  
 $\text{C}(\text{O})(\text{OR}^{13})$ ,  $\text{C}(\text{O})(\text{SR}^{13})$ ,  $\text{SR}^5$ ,  $\text{SSR}^7$  or  $\text{SSR}^5$ , or does not exist;  $\text{R}^6$ ,  $\text{R}^7$ ,  $\text{R}^8$ ,  $\text{R}^9$ ,  $\text{R}^{10}$ ,  $\text{R}^{11}$ ,  $\text{R}^{12}$ ,  $\text{R}^{13}$ ,  
10  $\text{R}^{14}$ ,  $\text{R}^{15}$ ,  $\text{R}^{16}$  are the same or different alkyl or acyl groups containing 1-24 carbon atoms which  
may contain 1-4  $\text{ONO}_2$  substituents; or  $\text{C}_1 - \text{C}_6$  connections to  $\text{R}^1 - \text{R}^4$  in cyclic derivatives; or  
are each independently hydrogen; a nitrate group; or A; M is H,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{NH}_4^+$ ,  $\text{N}^+\text{H}_k\text{R}^{11}_{(4-k)}$   
where k is 0-3, or other pharmaceutically acceptable counterion.

15 Preferred therapeutic compounds for use in the invention include compounds in which  
 $\text{R}^{19}$  is X-Y. In a particularly preferred embodiment:  $\text{R}^{19}$  is X-Y and  $\text{R}^5$ ,  $\text{R}^6$ ,  $\text{R}^8$ ,  $\text{R}^9$ ,  $\text{R}^{10}$ ,  $\text{R}^{12}$ ,  $\text{R}^{13}$ ,  
 $\text{R}^{14}$ ,  $\text{R}^{15}$ ,  $\text{R}^{16}$  are the same or different alkyl groups containing 1-24 carbon atoms which may  
contain 1-4  $\text{ONO}_2$  substituents, or  $\text{C}_1$  or  $\text{C}_2$  connections to  $\text{R}^1 - \text{R}^3$  in cyclic derivatives;  $\text{R}^1$   
and  $\text{R}^3$  are the same or different and selected from H,  $\text{C}_1 - \text{C}_4$  alkyl chains, which may include  
one O, linking  $\text{R}^1$  and  $\text{R}^3$  to form pentosyl, hexosyl, cyclopentyl, or cyclohexyl rings, which  
20 rings may optionally bear hydroxyl substituents;  $\text{R}^2$  and  $\text{R}^4$  are the same or different and  
selected from H, a nitrate group,  $\text{C}_1 - \text{C}_4$  alkyl optionally bearing 1-3 nitrate group, and acyl  
groups ( $-\text{C}(\text{O})\text{R}^5$ );  $\text{R}^7$ ,  $\text{R}^{11}$  are the same or different  $\text{C}_1 - \text{C}_8$ , alkyl or acyl..

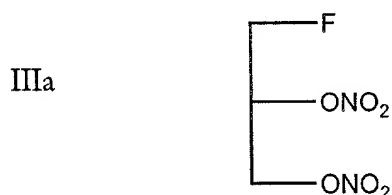
In certain embodiments in which  $\text{R}_{19}$  is X-Y, m, p = 1, and n = 0. In other  
embodiments in which  $\text{R}_{19}$  is X-Y, X is selected from  $\text{CH}_2$ , O, NH, NMe, CN,  $\text{NHOH}$ ,  
25  $\text{N}_2\text{H}_3$ ,  $\text{N}_2\text{H}_2\text{R}^{13}$ ,  $\text{N}_2\text{HR}^{13}\text{R}^{14}$ ,  $\text{N}_3$ , S, SCN,  $\text{SCN}_2\text{H}_2(\text{R}^{15})_2$ ,  $\text{SCN}_2\text{H}_3(\text{R}^{15})$ ,  $\text{SC}(\text{O})\text{N}(\text{R}^{15})_2$ ,  
 $\text{SC}(\text{O})\text{NHR}^{15}$ ,  $\text{SO}_3\text{M}$ ,  $\text{SH}$ ,  $\text{SR}^7$ ,  $\text{SO}_2\text{M}$ ,  $\text{S}(\text{O})\text{R}^8$ ,  $\text{S}(\text{O})_2\text{R}^9$ ,  $\text{S}(\text{O})\text{OR}^8$ ,  $\text{S}(\text{O})_2\text{OR}^9$ ,  $\text{PO}_3\text{HM}$ ,  
 $\text{PO}_3\text{M}_2$ ,  $\text{P}(\text{O})(\text{OR}^{15})(\text{OR}^{16})$ ,  $\text{P}(\text{O})(\text{OR}^{16})(\text{OM})$ ,  $\text{P}(\text{O})(\text{R}^{15})(\text{OR}^8)$ ,  $\text{P}(\text{O})(\text{OM})\text{R}^{15}$ ,  $\text{CO}_2\text{M}$ ,  $\text{CO}_2\text{H}$ ,  
 $\text{CO}_2\text{R}^{11}$ ,  $\text{C}(\text{O})$ ,  $\text{C}(\text{O})\text{R}^{12}$ ,  $\text{C}(\text{O})(\text{OR}^{13})$ ,  $\text{PO}_2\text{M}$ ,  $\text{P}(\text{O})(\text{OR}^{14})$ ,  $\text{P}(\text{O})(\text{R}^{13})$ ,  $\text{SO}$ ,  $\text{SO}_2$ ,  $\text{C}(\text{O})(\text{SR}^{13})$ ,  
 $\text{SSR}^4$ . In another embodiment in which  $\text{R}_{19}$  is X-Y, Y is selected from CN,  $\text{N}_2\text{H}_2\text{R}^{13}$ ,

$N_2HR^{13}R^{14}$ ,  $N_3$ ,  $SCN$ ,  $SCN_2H_2(R^{15})_2$ ,  $SC(O)N(R^{15})_2$ ,  $SC(O)NHR^{15}$ ,  $SO_3M$ ,  $SR^4$ ,  $SO_2M$ ,  
 $PO_3HM$ ,  $PO_3M_2$ ,  $P(O)(OR^{15})(OR^{16})$ ,  $P(O)(OR^{16})(OM)$ ,  $P(O)(R^{15})(OR^8)$ ,  $P(O)(OM)R^{15}$ ,  $CO_2M$ ,  
 $CO_2H$ ,  $CO_2R^{11}$ ,  $C(O)R^{12}$ ,  $C(O)(SR^{13})$ ,  $SR^5$ ,  $SSR^5$ , or does not exist. In a further  
embodiment, X and/or Y contain a sulfur-containing functional group. In certain preferred  
5 embodiments, the compound of the invention comprises a heterocyclic functionality, more  
preferably, a nucleoside or nucleobase. In further preferred embodiments, the compound of  
the invention comprises a carbocyclic functionality, more preferably, a steroidal or  
carbohydrate moiety.

In another aspect of the invention, a therapeutic compound of the invention is  
10 represented by the formula (Formula III):

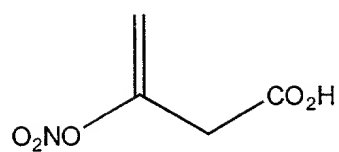


in which: m, n are 1-10;  $R^{1-18}$ , X, and Y have the meaning as defined above. In certain  
preferred embodiments,  $R^6 - R^{16}$  are the same or different alkyl or acyl groups containing 1-24  
carbon atoms which may contain 1-4  $ONO_2$  substituents, or  $C_1 - C_6$  connections to  $R^1 - R^4$  in  
cyclic derivatives. In certain preferred embodiments,  $R^{18}$  is A and  $m = n = 1$ . In further  
25 preferred embodiments, therapeutic compounds of the invention have a formula selected from  
(Formulae IIIa - IIIam):

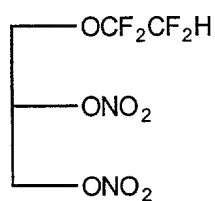




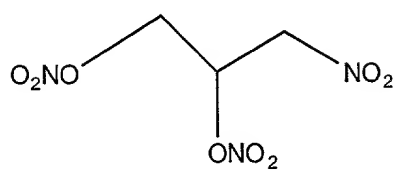
IIIb



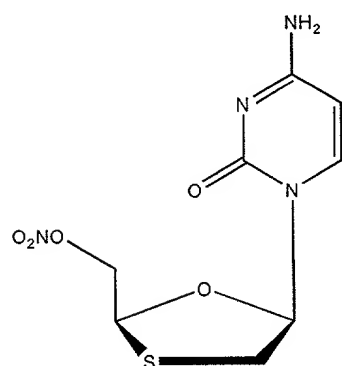
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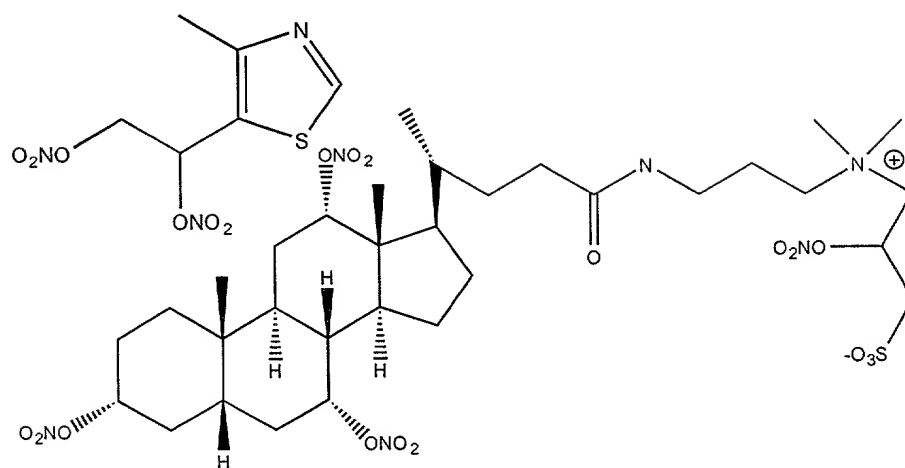
IIIId



IIIe

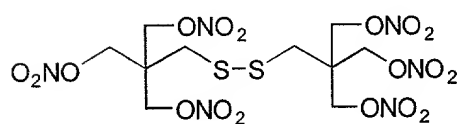


IIIIf

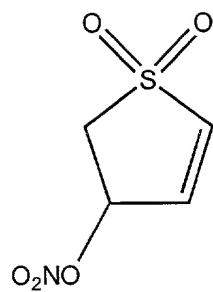


IIIg

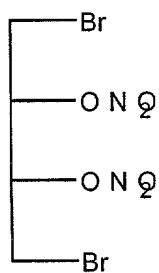
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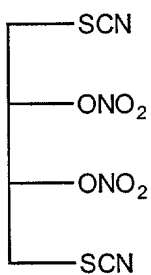
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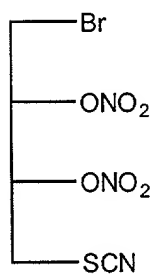
IIIj



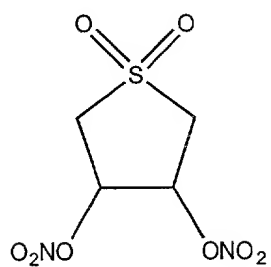
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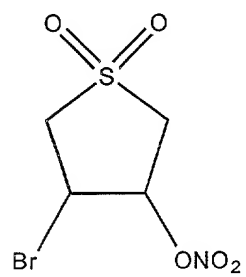
IIIl



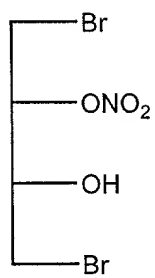
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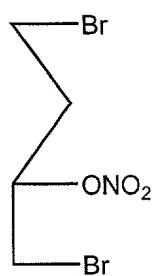
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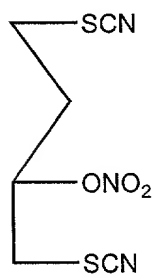
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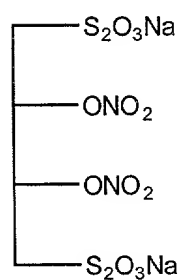
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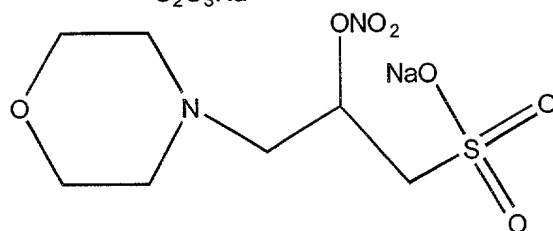
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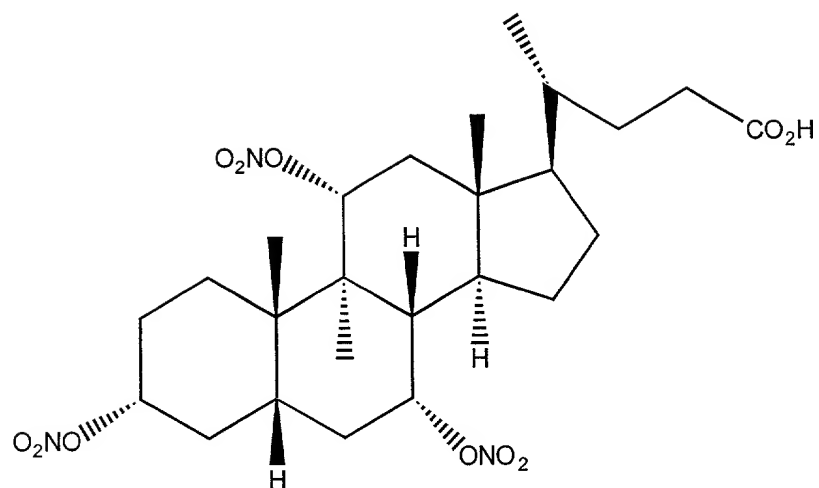


IIIr

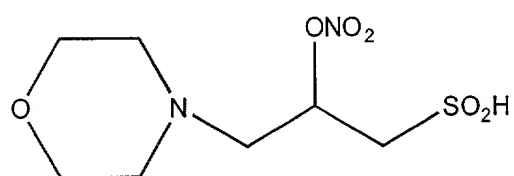


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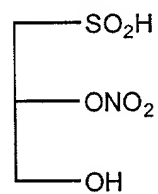


III<sub>t</sub>

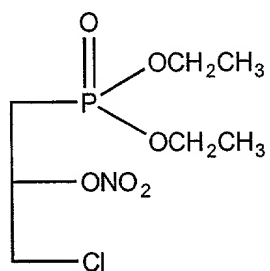
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III<sub>u</sub>

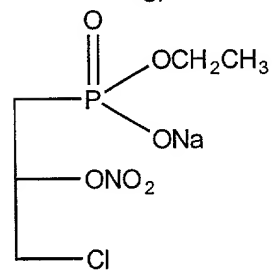
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III<sub>v</sub>

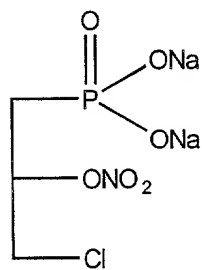
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III<sub>w</sub>

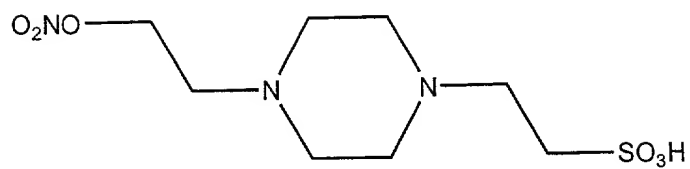
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III<sub>x</sub>

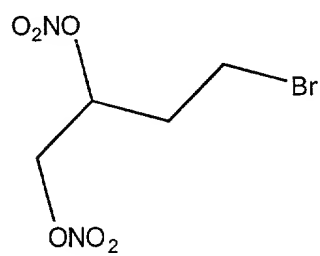
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III<sub>y</sub>

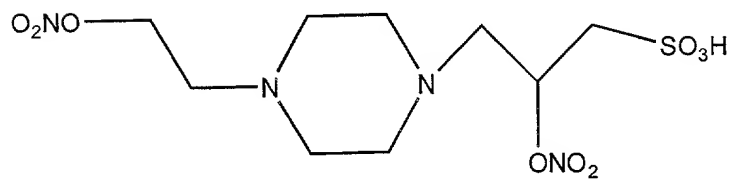
IIIz



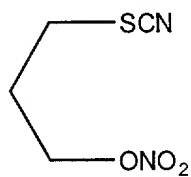
5 IIIaa



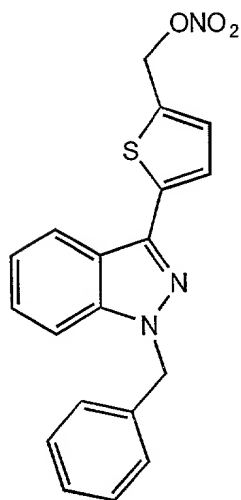
10 IIIab



15 IIIac

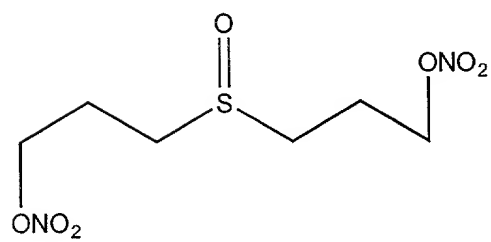


20 IIIad

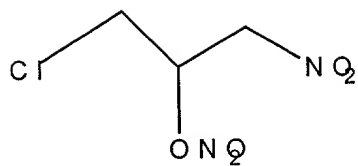


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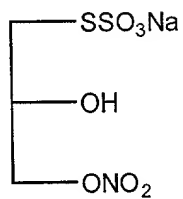


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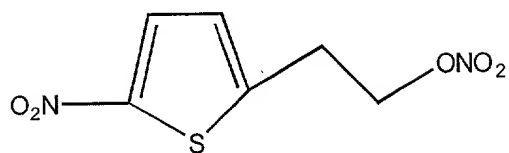


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IIIag

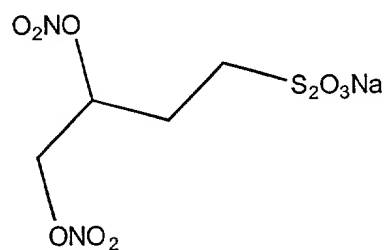


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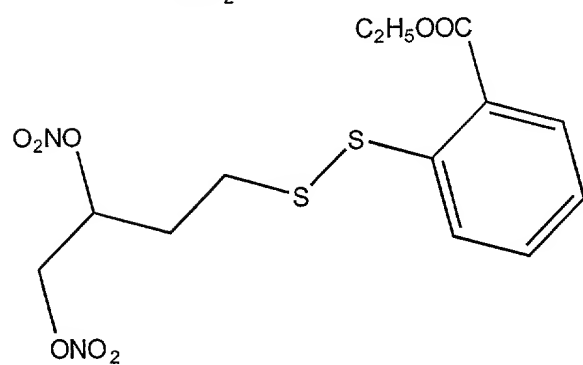
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IIIai



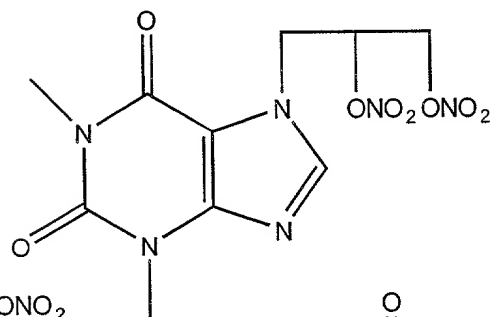
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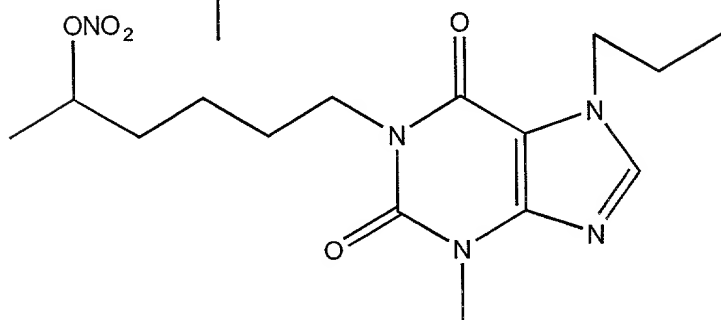
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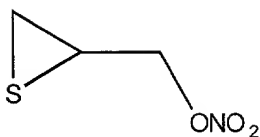


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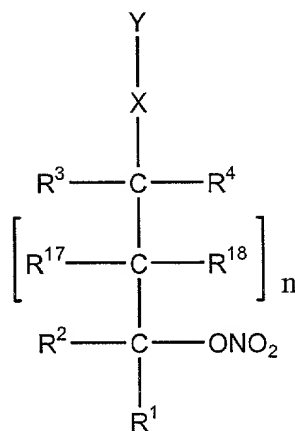
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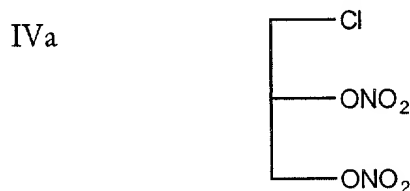


In a further aspect of the invention, a therapeutic compound of the invention is represented by the formula (Formula IV):

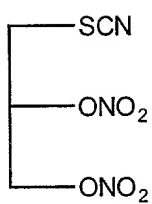


in which  $n = 0$ ,  $X$  is  $CH_2$  or does not exist, and  $Y$  is selected from,  $F$ ,  $Br$ ,  $Cl$ ,  $CH_3$ ,  $CF_2H$ ,  $CF_3$ ,  $OH$ ,  $NH_2$ ,  $NHR_6$ ,  $NR_6R_7$ ,  $CN$ ,  $NHOH$ ,  $N_2H_3$ ,  $N_2H_2R_{13}$ ,  $N_2HR_{13}R_{14}$ ,  $N_3$ ,  $S$ ,  $SCN$ ,  $SCN_2H_2(R_{15})_2$ ,  $SCN_2H_3(R_{15})$ ,  $SC(O)N(R_{15})_2$ ,  $SC(O)NHR_{15}$ ,  $SO_3M$ ,  $SH$ ,  $SR_7$ ,  $SO_2M$ ,  $S(O)R_8$ ,  $S(O)_2R_9$ ,  $S(O)OR_8$ ,  $S(O)_2OR_9$ ,  $PO_2HM$ ,  $PO_3M_2$ ,  $P(O)(OR_{15})(OR_{16})$ ,  $P(O)(OR_{16})(OM)$ ,  $P(O)(R_{15})(OR_8)$ ,  $P(O)(OM)R_{15}$ ,  $CO_2M$ ,  $CO_2H$ ,  $CO_2R_{11}$ ,  $C(O)R_{12}$ ,  $C(O)(OR_{13})$ ,  $C(O)(SR_{13})$ ,  $SR_5$ ,  $SSR_7$  or  $SSR_5$ .  $R_2$ ,  $R_4$ ,  $R_5$ ,  $R_6$ ,  $R_7$ ,  $R_8$ ,  $R_9$ ,  $R_{10}$ ,  $R_{11}$ ,  $R_{12}$ ,  $R_{13}$ ,  $R_{14}$ ,  $R_{15}$ , and  $R_{16}$  are as defined above. In certain preferred embodiments,  $R_2$  and  $R_4$  are optionally  $H$ , a nitrate group or a connection to  $R_5$ - $R_{16}$  in cyclic derivatives.

In certain preferred embodiments, a compounds of the invention is represented by the formula (Formulae IVa-IVt):

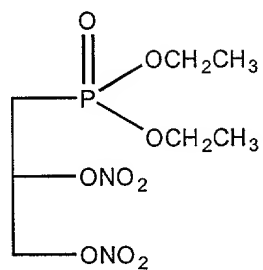


IVb



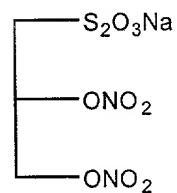
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IVc



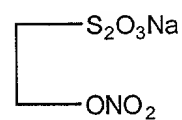
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IVd

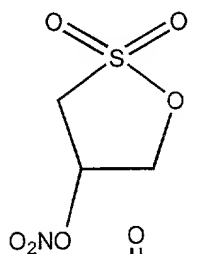


15

IVe

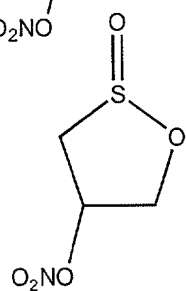


IVf



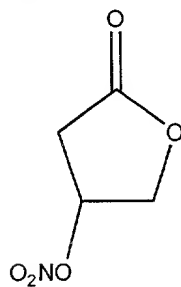
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IVg



25

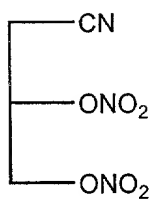
IVh





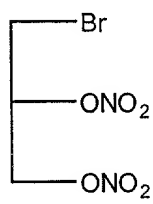
IVi

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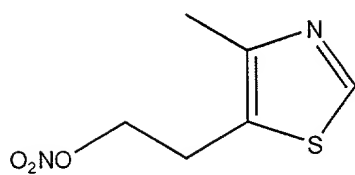
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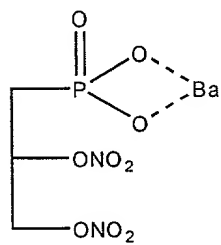
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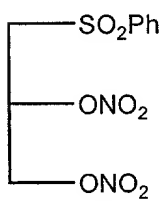
IVl

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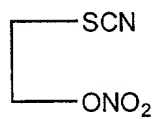


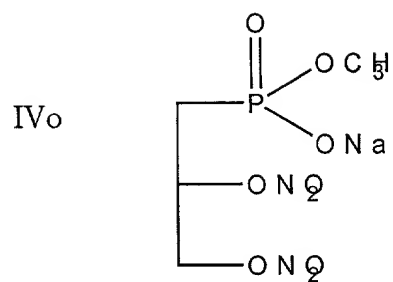
IVm

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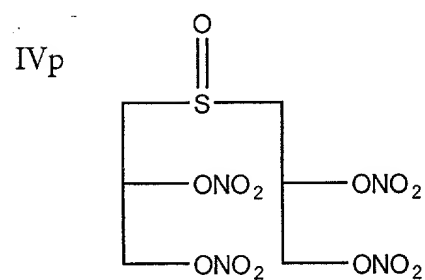


IVn

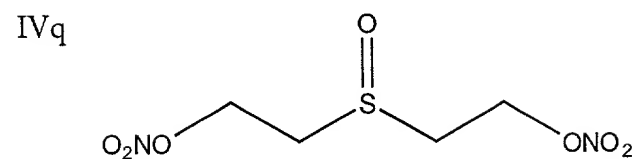




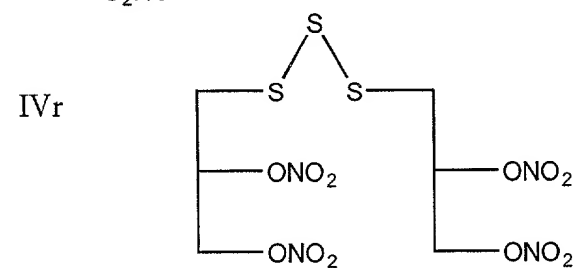
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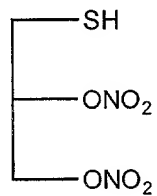
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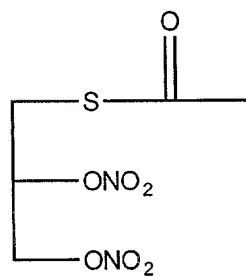
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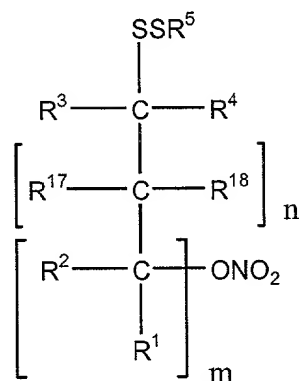
20 IVs



25 IVt



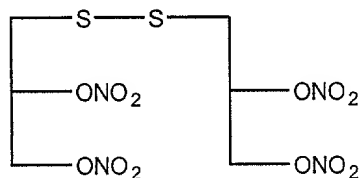
In a further aspect of the invention, compounds according to the invention are represented by the formula (Formula V):



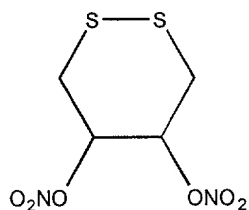
in which  $\text{R}_2$  is optionally H or a connection to  $\text{R}_5$  in cyclic derivatives,  $\text{R}_4$  is H or a nitrate group, and  $\text{R}_5$  is as described above.

In certain preferred embodiments, compounds of the invention are represented by the formula (Formulae Va-Vag):

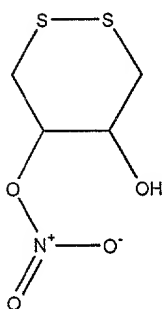
Va



Vb

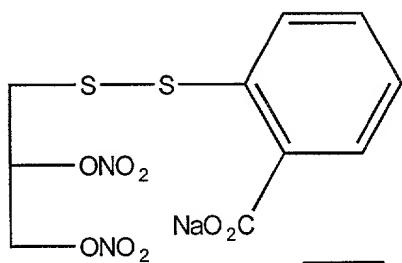


Vc



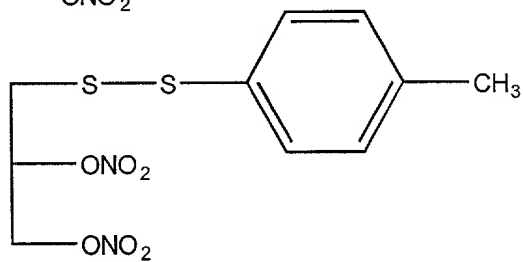
Vd

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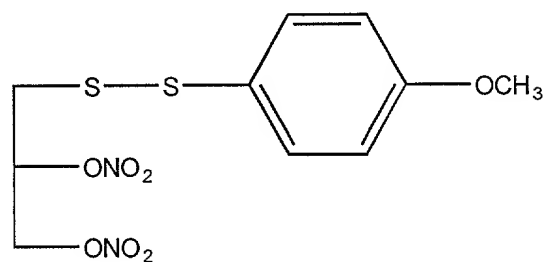
Ve

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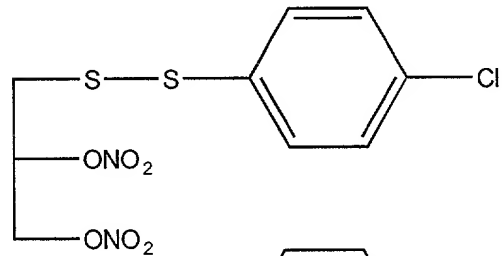
Vf

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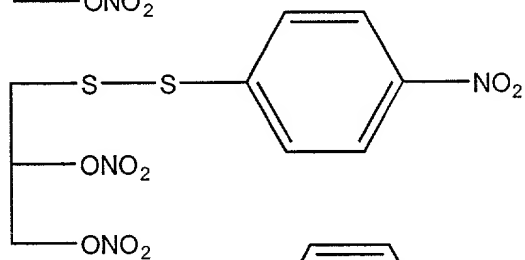
Vg

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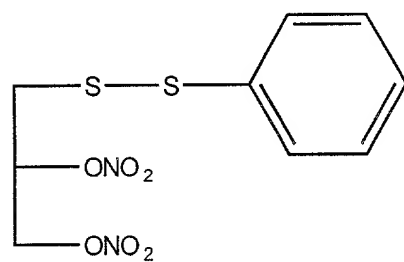
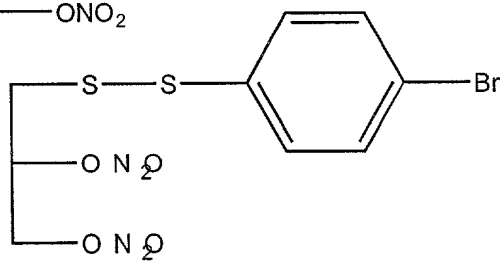
Vh

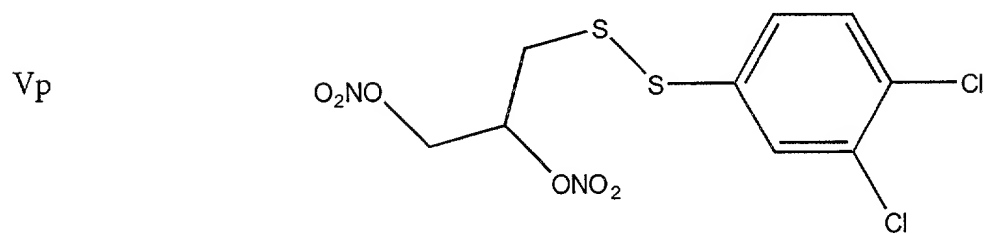
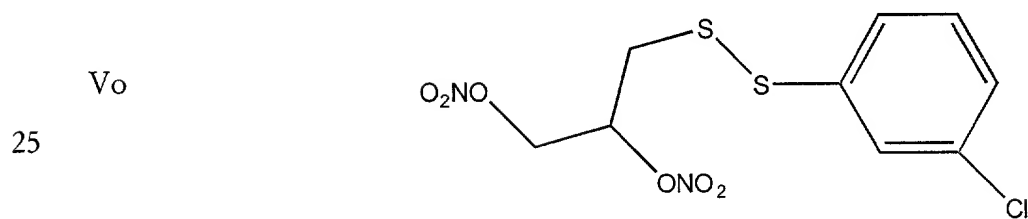
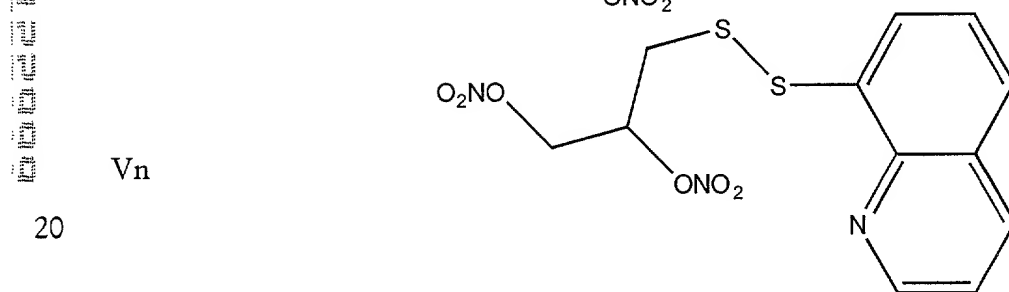
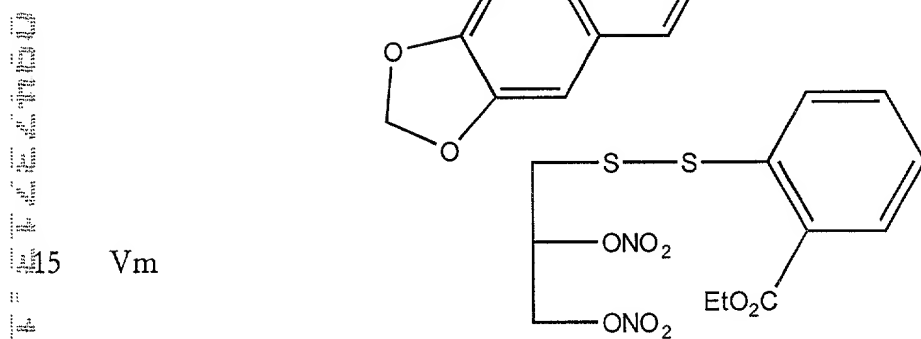
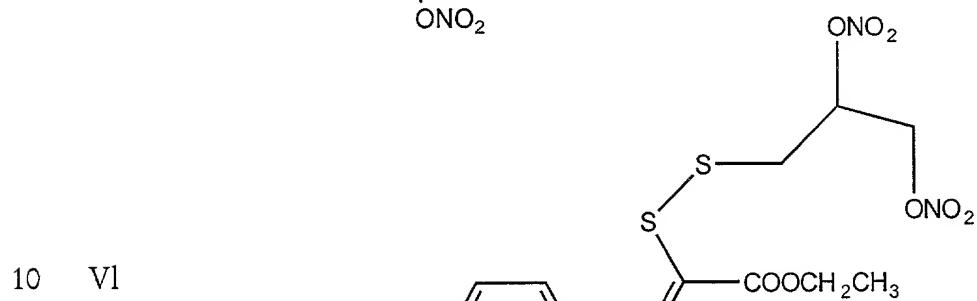
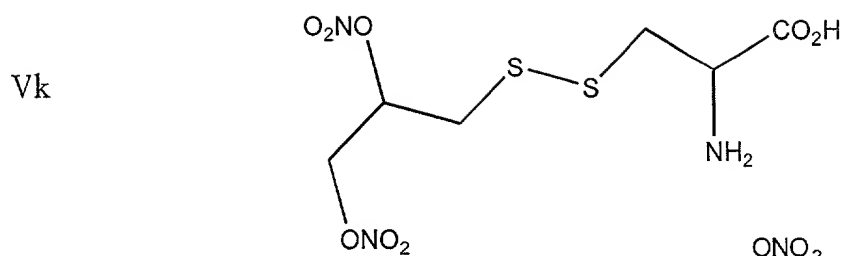
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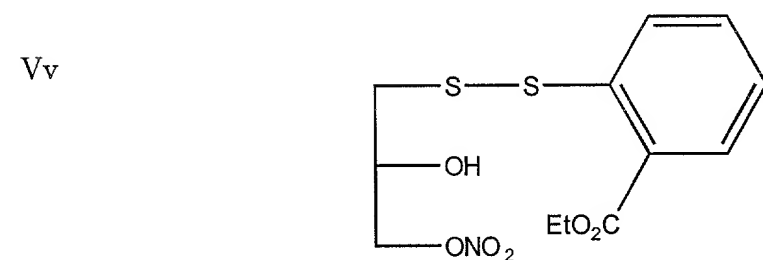
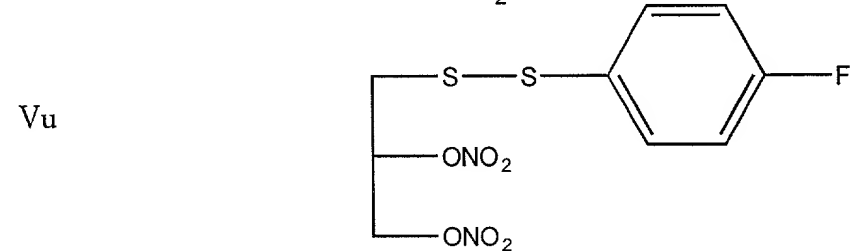
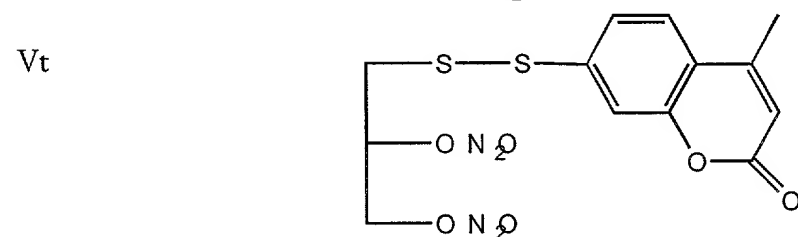
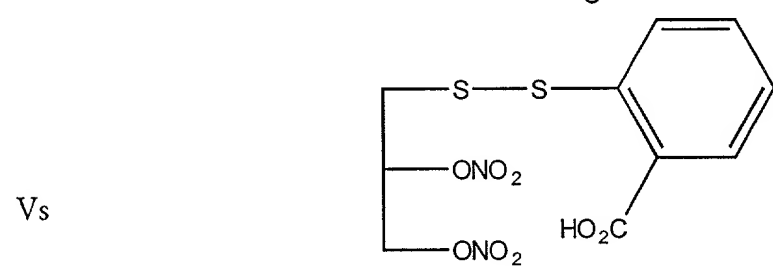
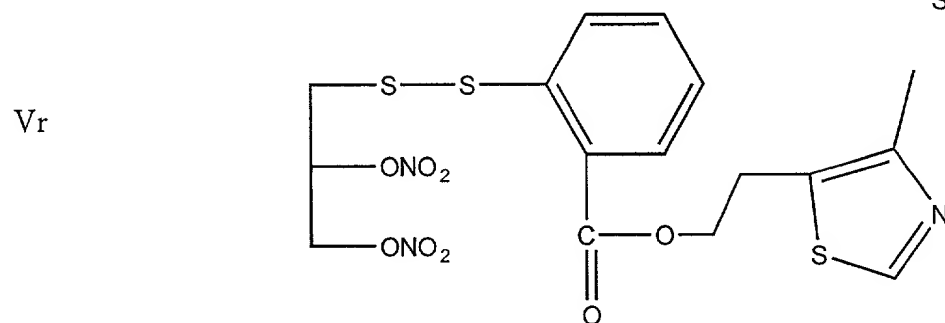
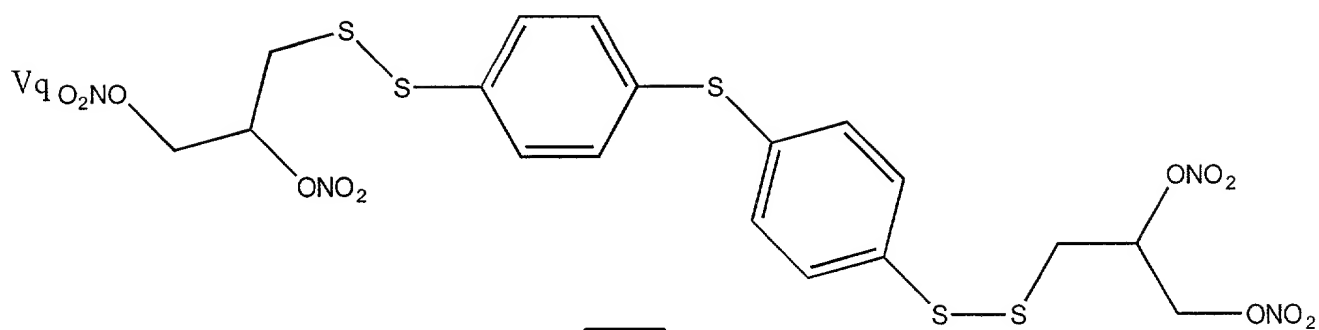


Vi

Vj

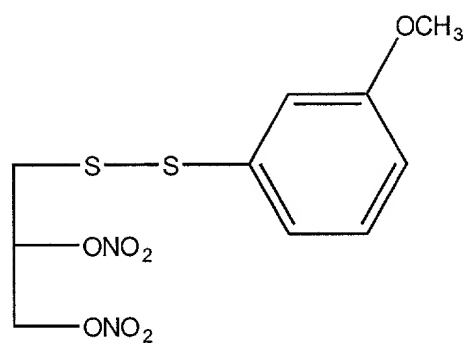




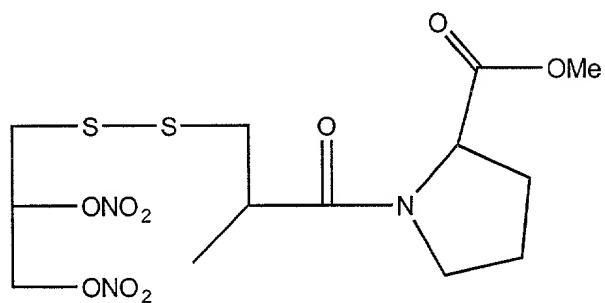


V<sub>w</sub>

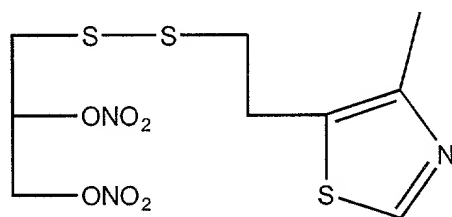
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V<sub>x</sub>

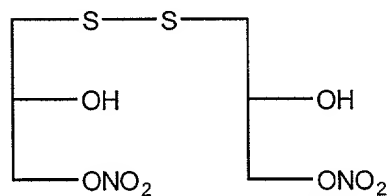
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V<sub>y</sub>

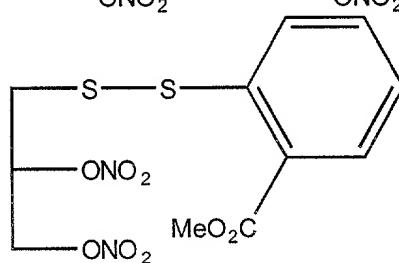
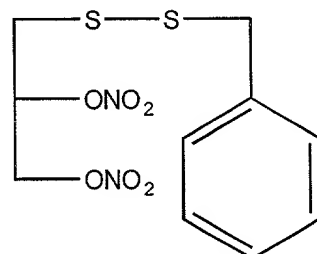
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V<sub>z</sub>

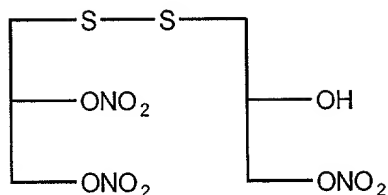
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V<sub>aa</sub>

25

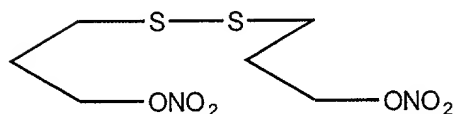
V<sub>ab</sub>

Vac



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Vad



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Vae

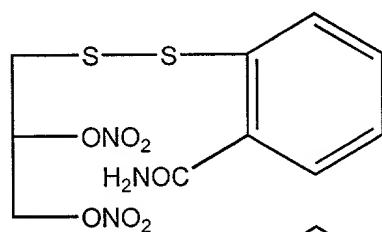
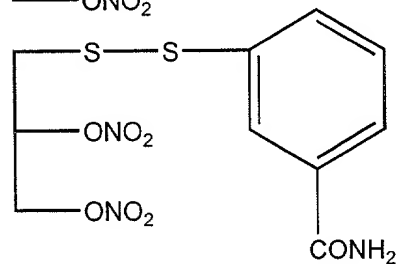


Table 1

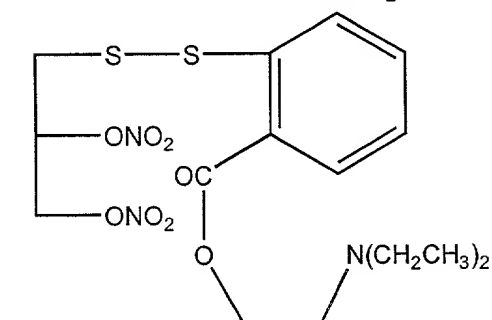
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Vaf



Vag

20



In another aspect, the invention provides novel compounds which can be represented by structures of Formula III, Formula IV, and Formula V. Table 1 lists data associated with these compounds using art-recognized characterization techniques. Further, the invention provides novel pharmaceutical compositions comprising a therapeutic compound (nitrate ester) of the invention and a pharmaceutically acceptable vehicle.



Table 1	$^1\text{H}$ NMR	$^{13}\text{C}$ NMR
IIIa	( $\text{CDCl}_3$ ): 5.34-5.57 (1H, dm, $^3J_{\text{HF}}$ 20.6), 4.53-4.87 (4H, superposition several multiplets, $\text{O}_2\text{NO}-\text{CH}_2 + \text{CH}_2\text{F}$ , $^2J_{\text{HF}}$ 46.7, $^4J_{\text{HF}}$ 0.66)	( $\text{CDCl}_3$ ): 79.47 (d, $^1J_{\text{CF}}$ 177), 76.73 (d, $^2J_{\text{CF}}$ 20.6), 67.84 (d, $^3J_{\text{CF}}$ 6.87)
IIIc	( $\text{CDCl}_3$ ): $\delta$ 5.7 (1H, t, $^2J_{\text{HF}}$ 54), 5.45 (1H, m), 4.5-4.9 (2H, m), 4.15-4.35 (1H, m)	( $\text{CDCl}_3$ ): $\delta$ 75.55, 68.05, 60.76
III d	( $\text{CDCl}_3$ ): $\delta$ 5.46 (1H, m), 4.80-4.87 (1H, dd, J 3.5, 12.9), 4.65-4.72 (1H, dd, J 6.2, 12.9), 3.7-3.8 (2H, m)	( $\text{CDCl}_3$ ): $\delta$ 77.24, 68.57, 39.86
III f	( $\text{CDCl}_3$ ) $\delta$ 8.72 (s, 1H), 5.38 (t, 1H), 4.6 (d, 2H), 2.45 (s, 3H)	-
III g	( $\text{DMSO-d}_6$ ) $\text{CHONO}_2$ only: $\delta$ 4.8-5.8	( $\text{DMSO-d}_6$ ) $\text{CONO}_2$ only: $\delta$ 85.68, 84.17, 82.47, 76.50
III h	( $\text{CD}_3\text{OD}$ ) $\delta$ 4.85 (3H, m), 3.5 (1H, m)	( $\text{CD}_3\text{OD}$ ) $\delta$ 70.61, 36.74
III i	( $\text{CDCl}_3$ ): $\delta$ 6.95 (dd, 1H), 6.71 (dd, 1H), 6.09 (m, 1H), 3.80 (dd, 1H), 3.32 (dd, 1H)	( $\text{CDCl}_3$ ): $\delta$ 137.9, 132.5, 76.6, 52.9
III j	( $\text{CDCl}_3$ ): $\delta$ 5.62 (2H, m), 3.60 (4H, m)	( $\text{CDCl}_3$ ): $\delta$ 77.87, 25.22
III k	( $\text{CD}_3\text{CN}$ ): $\delta$ 3.45 (m, 2H), 5.72 (m, 2H)	( $\text{CD}_3\text{CN}$ ): $\delta$ 79.98, 28.87
III l	-	( $\text{CD}_3\text{CN}$ ): $\delta$ 79.48, 33.45, 28.47
III m	( $\text{DMSO-d}_6$ ): $\delta$ 5.97 (m, 2H), 3.80 (m, 4H)	( $\text{DMSO-d}_6$ ): $\delta$ 78.84, 52.60
III n	( $\text{CDCl}_3$ ): $\delta$ 5.73 (m, 1H), 4.62 (m, 1H), 3.96-3.77 (m, 1H), 3.58-3.32 (m, 1H)	( $\text{CDCl}_3$ ): $\delta$ 81.47, 57.85, 53.50, 38.75
III o	-	( $\text{CDCl}_3$ ): $\delta$ 81.24, 69.79, 33.26, 27.24
III p	( $\text{CDCl}_3$ ): $\delta$ 5.36 (m, 1H), 3.11-3.60 (m, 4H), 2.33 (m, 2H)	( $\text{CDCl}_3$ ): $\delta$ 78.92, 33.66, 30.64, 27.36
III q	( $\text{CDCl}_3$ ): $\delta$ 5.47 (m, 1H), 3.53-3.05 (m, 4H), 2.29 (m, 2H)	( $\text{CDCl}_3$ ): $\delta$ 81.32, 37.12, 32.97, 30.98
III w $^{31}\text{P}(\text{CDCl}_3$ , 162 MHz) 24.60	( $\text{CDCl}_3$ , 300 MHz): 5.31-5.45 (m, 1H), 3.92-4.08 (m, 4H), 3.63-3.81 (m, 2H), 2.03-2.30 (m, 2H), 1.16-1.24 (superposition of 2 t, 6H, J 7)	( $\text{CDCl}_3$ , 75 MHz): 76.83, 62.15 (d, J 6.37), 43.77 (d, J 8.95), 27.08 (d, J 142.00), 15.99 (d, J 5.88)

IIIx <sup>31</sup> P (CD <sub>3</sub> OD, 122 MHz) 17.62	(CD <sub>3</sub> OD, 300 MHz): 5.38-5.63 (m, 1H), 3.75-4.25 (superposition of 2 m, 4H), 1.88-2.20 (m, 2H), 1.12-1.28 (t, 3H)	(CD <sub>3</sub> OD, 75 MHz): 81.14, 61.17 (d, J 5.41), 45.56 (d, J 5.94), 29.35 (d, J 131.74), 17.00 (d, J 6.75)
IIIak	<sup>1</sup> H (CD <sub>3</sub> CN, 300 MHz): 7.8 (s, 1H), 5.75-5.85 (m, 1H), 4.90-4.97 (dd, 1H, J 12.87, 3.39), 4.54-4.76 (m, 3H), 3.46 (s, 3H), 3.27 (s, 3H)	<sup>13</sup> C (CD <sub>3</sub> CN, 75 MHz) (for polynitrated chain): 78.51, 70.58, 46.01, 30.10, 28.18
Ivi	(CDCl <sub>3</sub> ): δ 5.45 (1H, m), 4.83 (1H, dd), 4.65 (1H, dd), 2.9 (2H, m)	(CD <sub>3</sub> OD): δ 116.44, 75.37, 71.20, 19.19
Ivk	(CDCl <sub>3</sub> ): δ 8.55 (s, 1H), 4.55 (t, 2H), 3.15 (t, 2H), 2.37 (s, 3H)	(CDCl <sub>3</sub> ): δ 150.9, 150.7, 125.3, 72.53, 24.47, 15.18
Ivm	(CDCl <sub>3</sub> ): δ 7.5-8.0 (arom, 5H), 5.7 (1H, m), 4.94 (1H, dd), 4.62 (1H, dd), 3.5 (2H, m)	(CDCl <sub>3</sub> ): δ 135.45, 134.79, 129.81, 27.95, 73.08, 70.04, 54.73
Ivs	<sup>1</sup> H-NMR(CDCl <sub>3</sub> , 300 MHz): 5.23-5.32 (1H, m), 4.87 (1H, dd, J 12.82, 3.22), 4.68 (1H, dd, J 12.83, 6.09), 2.77-2.94 (2H, m), 1.66 (1H, t, J 9.07)	<sup>13</sup> C-NMR: (CDCl <sub>3</sub> , 75.48 MHz): 79.39, 69.30, 23.68
Ivt	<sup>1</sup> H-NMR(CDCl <sub>3</sub> , 300 MHz): 5.29-5.38 (1H, m), 4.76 (1H, dd, J 12.94, 3.11), 4.55 (1H, dd, J 12.94, 6.37), 3.30 (1H, dd, J 14.06, 5.98), 3.13 (1H, dd, J 14.61, 6.35)	<sup>13</sup> C-NMR: (CDCl <sub>3</sub> , 75.48 MHz): 194.10, 77.00, 69.79, 30.42, 27.78
Vb	(CDCl <sub>3</sub> ): δ 5.56 (m, 2H), 3.38-2.95 (m, 4H)	(CD <sub>3</sub> OD): δ 85.93, 32.77
Vc	(CDCl <sub>3</sub> ): δ 5.85-5.91 (1H, m), 4.50-4.58 (1H, m), 3.22-3.29 (1H, dd, J 5.47, 12.78), 2.97-3.05 (1H, dd, J 4.6, 11.88), 2.82-2.90 (1H, dd, J 2.87, 12.78), 2.74-2.83 (1H, dd, J 3.15, 11.9)	(CDCl <sub>3</sub> ): δ 87.6, 74.96, 36.20, 31.54
Ve	(CDCl <sub>3</sub> ): δ 7.44-7.51 (m, arom 2H), 7.17-7.24 (d, arom 2H, J 7.91), 5.47-5.59 (m, 1H), 4.83-4.93 (dd, 1H, J 12.81, 2.78), 4.57-4.67 (dd, 1H, J 12.82, 5.71), 3.02-3.12 (dd, 1H, J 14.48, 6.01), 2.9-2.99 (dd, 1H, J	(CDCl <sub>3</sub> ): δ 21.53, 36.78, 69.82, 77.68, 130.52, 130.62, 132.55, 139.23

	14.47, 7.72), 2.38 (s, 3H)	
Vf	(CDCl <sub>3</sub> ): δ 7.48-7.57 (m, arom 2H), 7.48-7.57 (m, arom 2H), 5.49-5.59 (m, 1H), 4.84-4.93 (dd, 1H, J 12.79, 2.79), 4.58-4.68 (dd, 1H, J 12.79, 5.75), 3.84 (s, 3H), 3.02-3.12 (dd, 1H, J 14.47, 5.8), 2.89-2.99 (dd, 1H, J 14.46, 7.99).	(CDCl <sub>3</sub> ): δ 36.57, 55.87, 69.75, 77.76, 115.47, 126.71, 133.76, 160.94.
Vg	(CDCl <sub>3</sub> ): δ 7.47-7.54 (m, arom 2H), 7.32-7.38 (m, arom 2H), 5.45-5.55 (m, 1H), 4.84-4.97 (dd, 1H, J 12.86, 2.92), 4.58-4.68 (dd, 1H, J 12.86, 5.68), 2.91-3.11 (m, 2H).	(CDCl <sub>3</sub> ): δ 36.87, 69.80, 77.5, 129.98, 130.85, 134.51, 134.79.
Vh	(CDCl <sub>3</sub> ): δ 8.21-8.27 (m, arom 2H), 7.67-7.74 (m, arom 2H), 5.44-5.54 (m, 1H), 4.86-4.94 (dd, 1H, J 12.92, 3.11), 4.61-4.70 (dd, 1H, J 12.92, 5.56), 3.01-3.16 (m, 2H).	(CDCl <sub>3</sub> ): δ 37.24, 69.75, 77.41, 124.82, 127.26, 144.83
Vi	(CDCl <sub>3</sub> ): δ 7.40-7.55 (m, arom 4H), 5.44-5.55 (m, 1H), 4.85-4.92 (dd, 1H, J 12.87, 2.91), 4.60-4.70 (dd, 1H, J 12.86, 5.66), 2.92-3.11 (m, 2H).	(CDCl <sub>3</sub> ): δ 36.87, 69.79, 77.48, 122.72, 130.93, 132.90, 135.15.
Vj	(CDCl <sub>3</sub> ): δ 7.55-7.62 (d, arom 2H, J 7.16), 7.29-7.44 (m, arom 3H), 5.46-5.58 (m, 1H), 4.82-4.92 (dd, 1H, J 12.85, 2.79), 4.57-4.67 (dd, 1H, J 12.86, 5.67), 3.01-3.13 (dd, 1H, J 14.51, 6.24), 2.92-3.02 (dd, 1H, J 14.52, 7.4)	(CDCl <sub>3</sub> ): δ 36.97, 69.88, 77.61, 128.60, 129.50, 129.85, 136.02
Vk	(CDCl <sub>3</sub> , 300 MHz): 8.09 (1H, dd, J 8.12, 0.36), 8.02 (1H, dd, J 7.8, 1.15), 7.51-7.59 (1H, m, J 7.24, 1.44), 7.21-7.29 (1H, m, J 7.35, 0.54), 5.40-5.49 (1H, m),	(CDCl <sub>3</sub> , 75.48 MHz): 166.21, 140.41, 132.78, 131.46, 127.63, 125.48, 77.25, 71.03, 61.42, 32.51, 28.29, 14.17

	4.70-4.78 (1H, dd, J 13.04, 2.95), 4.33-4.45 (3H, m, superposition of 1H from CH <sub>2</sub> -ONO <sub>2</sub> and quartet from O-CH <sub>2</sub> -CH <sub>3</sub> ), 2.66-2.87 (2H, quartet, J 6.92), 1.39 (3H, t, J 7.14)	
VI	(CDCl <sub>3</sub> , 300MHz): δ 7.97 (s, 1H), 7.48-7.51 (d, 1H), 7.17-7.22 (m, 1H), 6.84-6.89 (d, 1H), 6.05 (s, 2H), 5.48-5.58(m, 1H), 4.81-4.89 (dd, 1H), 4.53-4.61 (dd, 1H), 4.25-4.4 (m, 2H), 3.05-3.10 (m, 2H), 1.35-1.45 (t, 3H).	(CDCl <sub>3</sub> , 75.48 MHz): δ 14.17, 36.27, 62.15, 69.71, 76.57, 101.77, 108.27, 110.31, 125.20, 127.55, 127.87, 146.87, 147.811, 149.76, 165.89
Vm	(CDCl <sub>3</sub> , 300MHz): δ 8.04-8.11 (m, arom 2H), 7.55-7.62 (m, arom 1H), 7.30-7.34 (m, arom 1H), 5.43-5.54 (m, 1H), 4.88-4.97 (dd, 1H, J 12.95, 2.79), 4.62-4.71 (dd, 1H, J 12.94, 5.35), 4.45-4.39 (q, 2H, J 7.12), 2.92-3.08 (m, 2H), 1.39-1.47(t, 3H, J 7.13)	(CDCl <sub>3</sub> , 75.48 MHz): δ 14.17, 26.23, 35.95, 61.55, 69.54, 77.24, 125.56, 125.92, 127.91, 131.52, 132.93, 139.56, 166.189
Vn	(CDCl <sub>3</sub> , 300MHz): δ 8.92-8.97 (m, arom 1H), 8.07-8.23 (m, arom 2H), 7.46-7.77 (m, arom 3H), 5.53-5.62 (m, 1H), 4.91-4.99 (dd, 1H, J 12.98, 2.77), 4.61-4.7 (dd, 1H, J 12.98, 5.35), 3- 3.18 (m, 2H)	(CDCl <sub>3</sub> , 75.48 MHz): δ 36.06, 69.62, 70.24, 77.42, 121.97, 125.65, 126.43, 126.70, 128.52, 135.31, 136.37, 145.62, 149.65
Vo	(CDCl <sub>3</sub> , 300MHz): δ 7.55-7.59 (m, arom 1H), 7.40-7.45 (m, arom 1H), 7.25-7.36 (m, arom 2H), 5.45-5.55 (m, 1H), 4.85-4.95 (dd, 1H, J 12.98, 3), 4.59-4.69 (dd, 1H, J 12.89, 6.67), 2.95-3.12 (m, 2H)	(CDCl <sub>3</sub> , 75.48 MHz): δ 37.10, 69.87, 77.44, 126.80, 128.38, 128.52, 130.83, 135.76, 138.01
Vp	(CDCl <sub>3</sub> , 300MHz): δ 7.65-7.67 (d, arom 1H J 2.15), 7.44- 7.47 (d, arom 1H, J 7.42), 7.28- 7.4 9 (m, arom 1H), 5.46-5.54 (m, 1H), 4.87- 4.94 (dd, 1H, J 12.89, 2.99), 4.61-4.68 (dd, 1H, J 12.88, 5.65), 2.95-3.12 (m, 2H)	(CDCl <sub>3</sub> 75.48 MHz): δ 36.59, 69.32, 76.90, 127.49, 129.86, 131.04, 132.31, 133.59, 135.65
Vq	(CDCl <sub>3</sub> , 300MHz): δ 7.2-7.6 (m, arom 8H), 5.42-5.56 (m, 2H), 4.82-4.95 (dd, 2H), 4.55- 4.67 (dd, 2H), 2.93-3.15 (m, 4H)	

Vr	(CDCl <sub>3</sub> , 300MHz): $\delta$ 8.63 (s, arom 1H), 8.01-8.13 (m, arom 2H), 7.57-7.65 (m arom 1H), 7.26- 7.37 (m, arom 1H), 5.42- 5.51 (m, arom 1H), 4.88-4.97 (dd, 1H, J 12.9, 2.7), 4.62-4.71 (dd, 2H, J 12.9, 5.1), 4.49- 4.57 (t, 2H, J 6.6), 3.23-3.32 (t, 2H, J 6.6), 2.91-3.08 (m, 2H), 2.45 (s, 3H)	
Vs	(d <sub>6</sub> -acetone), 300 MHz): 8.16-8.23 (1H, d, J 7.72), 8.07-8.13 (1H, dd, J 7.72, 1.11), 7.63-7.72 (1H, m, J 8.38, 1.26), 7.33-7.42 (1H, m, J 7.33), 5.67-5.76 (1H, m), 5.09-5.17 (1H, dd, J 12.91, 2.68), 4.83-4.93 (1H, dd, J 12.91, 5.91), 3.18-3.32 (2H, m)	(d <sub>6</sub> -acetone, 100.62 MHz): 167.74, 134.01, 132.64, 126.62, 126.21, 78.90, 71.69, 36.79
Vt	(CDCl <sub>3</sub> , 300MHz): $\delta$ 7.53-7.59 (d, arom 1H, J 8.35), 7.46-7.49 (d, arom 1H, J 1.82), 7.37-7.43 (m, arom 1H), 6.24 (s, 1H), 5.46-5.55 (m, 1H), 4.86-4.94 (dd, 1H, 12.92, 2.92), 4.61-4.69 (dd, 1H, J 12.93, 5.75), 3.0-3.15 (m, 2H), 2.42 (s, 3H)	(CDCl <sub>3</sub> , 75.48 MHz): $\delta$ 19.01, 37.10, 70.01, 77.46, 115.27, 115.34, 119.52, 123.07, 125.83, 141.13, 152.38, 154.25, 160.51
Vu	(CDCl <sub>3</sub> , 300MHz): $\delta$ 7.55-7.58 (m, arom 1H), 7.40-7.45 (m, arom 1H), 7.27-7.32 (m, arom 2H), 5.46-5.54 (m, 1H), 4.86-4.94 (dd, 1H, J 12.88, 2.92), 4.59-4.68 (dd, 1H, J 12.88, 5.65), 2.95-3.13 (m, 2H)	(CDCl <sub>3</sub> , 75.48 MHz): $\delta$ 37.08, 69.83, 77.40, 126.80, 128.40, 128.54, 130.83, 135.78, 137.98
Vv	(CDCl <sub>3</sub> , 300 MHz): 8.09-8.14 (1H, d, J 8.15), 7.98-8.04 (1H, dd, J 7.75, 0.91), 7.52-7.59 (1H, m, J 8.22, 1.19), 7.22-7.28 (1H, m, J 7.65), 4.53-4.61 (1H, dd, J 11.33, 3.63), 4.42-4.50 (1H, m, J 11.33, 6.27), 4.32-4.41 (2H, qu., J 7.13), 4.16-4.25 (1H, m), 2.74-2.84 (2H, m), 2.55-2.73 (1H, br.s.), 1.34-1.42 (3H, t, J 7.13)	(CDCl <sub>3</sub> , 75.48 MHz): 166.34, 140.25, 132.90, 131.49, 127.67, 125.60, 74.34, 66.83, 61.53, 40.89, 14.20
Vx	(CDCl <sub>3</sub> , 300MHz): $\delta$ 5.49-5.61 (m, 1H), 4.88-4.90 (m, 1H), 4.62-4.74 (m, 1H), 4.40-4.55 (m, 1H), 3.59-3.78 (m, superpos. 5H), 2.69-3.21 (m, superpos. 5H), 2.14-2.29 (m, 1H), 1.90-2.12 (m, 3H), 1.22-1.28	(CDCl <sub>3</sub> , 75.48 MHz): $\delta$ 17.39, 25.20, 29.40, 36.41/36.51, 38.55, 41.94/42.19, 47.32, 52.56, 59.12, 69.90/70.29, 77.89/78.00, 173.01, 173.48

	(d, 3H)	
Vy	(CDCl <sub>3</sub> , 300 MHz): $\delta$ 8.5 (s, 1H), 2.94-3.02 (t, 2H), 2.62-2.72 (q, 2H), 2.33 (s, 3H), 1.38-1.45 (t, 1H).	(CDCl <sub>3</sub> , 75.48 MHz): $\delta$ 15.41, 26.41, 31.51, 39.72, 129.36, 149.90
Vz	(CD <sub>3</sub> CN, 300 MHz): 4.60-4.68 (1H, dd, J 11.44, 3.36), 4.43-4.52 (1H, dd, J 11.35, 6.97), 4.11-4.21 (1H, m), 2.80-3.00 (2H, m)	(CD <sub>3</sub> CN, 75.48 MHz): 76.33, 67.34, 42.74
Vaa	(CDCl <sub>3</sub> , 300MHz): $\delta$ 8.05-8.15 (m, arom 2H), 7.55-7.65 (m, arom 1H), 7.25-7.35 (m, arom 1H), 5.40-5.55 (m, 1H), 4.85-4.95 (dd, 1H), 4.60-4.70 (dd, 1H), 3.95 (s, 3H), 2.90-3.10 (m, 2H)	(CDCl <sub>3</sub> , 5.48 MHz): $\delta$ 36.42, 52.87, 69.98, 77.69, 126.07, 126.45, 128.01, 132.06, 133.53, 140.161, 167.10
Vab	(CDCl <sub>3</sub> , 300MHz): $\delta$ 7.30-7.39 (m, arom 5H), 5.28-5.37 (m, 1H), 4.72-4.78 (dd, 1H, J 12.86, 2.79), 4.45-4.53 (dd, 1H, J 12.86, 5.9), 3.95 (s, 2H), 2.44-2.52 (dd, 1H, J 14.38, 6.1), 2.33-2.42 (dd, 1H, J 14.38, 7.34)	(CDCl <sub>3</sub> , 75.48 MHz): $\delta$ 32.01, 35.997, 43.66, 69.89, 77.72, 128.33, 129.22, 129.81, 137.22
Vac	<sup>1</sup> H-NMR(CDCl <sub>3</sub> , 300 MHz): 5.48-5.58 (1H, m), 4.89 (1H, ddd, J 12.91, 2.91, 1.21), 4.56-4.70 (2H, m), 4.48 (1H, dd, J 11.41, 6.4), 4.16-4.26 (1H, m), 2.94-3.12 (2H, m), 2.81-2.91 (2H, m)	<sup>13</sup> C-NMR: (CDCl <sub>3</sub> , 75.48 MHz): 77.30, 74.22, 69.46, 69.38, 66.90, 66.78, 42.12, 41.99, 36.82, 36.59
Vad	(CDCl <sub>3</sub> , 400 MHz): 5.55-5.65 (m, 1H), 4.87-4.94 (dd, 1H, J 12.94, 2.94), 4.62-4.70 (m, 1H, J 12.88), 3.13-3.30 (m, 2H)	(CDCl <sub>3</sub> , 400 MHz): 76.74, 69.46/69.42, 36.65/36.63

It will be noted that the structure of some of the compounds of this invention include asymmetric carbon atoms. It is to be understood accordingly that the isomers (e.g., enantiomers, diastereomers) arising from such asymmetry are included within the scope of this invention. Such isomers can be obtained in substantially pure form by classical separation techniques and by asymmetric synthesis (for example, see below in Example 21). Unless

expressly noted to the contrary, compounds referred to herein shall be construed to include both the R and S stereoisomers at each stereogenic centre.

In certain embodiments, a therapeutic compound of the invention comprises a cation (i.e., in certain embodiments, one of X or Y includes a cation, e.g., in the compound of formula IVd). If the cationic group is a proton, then the compound is considered an acid. If the proton is replaced by a metal ion or its equivalent, the compound is a salt.

Pharmaceutically acceptable salts of the therapeutic compound are within the scope of the invention. For example, M can be a pharmaceutically acceptable alkali metal (e.g., Li, Na, K), ammonium, alkaline earth metal (e.g., Ca, Ba, Mg), higher valency cation, or polycationic counter ion (e.g., polyammonium cation) (see e.g., Berge *et al.* (1977)). It will be appreciated that the stoichiometry of an anionic portion of the compound to a salt-forming cation will vary depending on the charge of the anionic portion of the compound and the charge of the counterion. Preferred pharmaceutically acceptable salts include a sodium, potassium, or calcium salt, but other salts are also contemplated within their pharmaceutically acceptable range.

Therapeutic compounds of the invention can be administered in a pharmaceutically acceptable vehicle. As used herein “pharmaceutically acceptable vehicle” includes any and all solvents, excipients, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like which are compatible with the activity of the compound and are physiologically acceptable to the subject. An example of a pharmaceutically acceptable vehicle is buffered normal saline (0.15 M NaCl). The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the therapeutic compound, use thereof in the compositions suitable for pharmaceutical administration is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Carrier or substituent moieties useful in the present invention may also include moieties which allow a therapeutic compound to be selectively delivered to a target organ. For example, delivery of a therapeutic compound to the brain may be enhanced by a carrier moiety using either active or passive transport (a “targeting moiety”). Illustratively, the

carrier molecule may be a redox moiety, as described in, for example, U.S. Patents 4,540,654 and 5,389,623, both to Bodor. These patents disclose drugs linked to dihydropyridine moieties which can enter the brain, where they are oxidized to a charged pyridinium species which is trapped in the brain. Thus drugs accumulate in the brain. Other carrier moieties include compounds, such as amino acids or thyroxine, which can be passively or actively transported *in vivo*. Such a carrier moiety can be metabolically removed *in vivo*, or can remain intact as part of an active compound. Structural mimics of amino acids (and other actively transported moieties) including peptidomimetics, are also useful in the invention. As used herein, the term "peptidomimetic" is intended to include peptide analogs which serve as appropriate substitutes for peptides in interactions with e.g., receptors and enzymes. The peptidomimetic must possess not only affinity, but also efficacy and substrate function. That is, a peptidomimetic exhibits functions of a peptide, without restriction of structure to amino acid constituents. Peptidomimetics, methods for their preparation and use are described in Morgan *et al.*, (1989) "Approaches to the discovery of non-peptide ligands for peptide receptors and peptidases". In *Annual Reports in Medicinal Chemistry* (Vinick, F.J., ed.) pp. 243-252, Academic Press, San Diego, CA. Many targeting moieties are known, and include, for example, asialoglycoproteins (see e.g., Wu, U.S. Patent 5,166,320) and other ligands which are transported into cells via receptor-mediated endocytosis (see below for further examples of targeting moieties which may be covalently or non-covalently bound to a target molecule).

In the methods of the invention, pain and/or inflammation in a subject is mitigated by administering an analgesic, sedative or anti-inflammatory therapeutic compound of the invention to the subject. The term "subject" is intended to include living organisms in which pain can occur. Examples of subjects include humans, apes, monkeys, cows, sheep, goats, dogs, cats, mice, rats, and transgenic species thereof. Administration of the compositions of the present invention to a subject to be treated can be carried out using known procedures, at dosages and for periods of time effective to alleviate pain in the subject. An effective amount of the therapeutic compound necessary to achieve a therapeutic effect may vary according to factors such as the subject, the age, sex, and weight of the subject, and the ability of the therapeutic compound to mitigate pain and inflammation in the subject. Dosage regimens can



be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. A non-limiting example of an effective dose range for a therapeutic compound of the invention (e.g., Va) is between 0.5 and 5000 mg/kg of body weight/per day, preferably between 50 and 1000 mg/kg/day, and still more preferably between 250 and 750 mg/kg/day. In an aqueous composition, preferred concentrations for the active compound (i.e., the therapeutic compound that can mitigate pain) are between 5 and 500 mM, more preferably between 10 and 100 mM, and still more preferably between 20 and 50 mM.

According to the invention, therapeutic compounds are administered to a subject by a route which is effective for mitigating inflammation, effecting analgesia and/or effecting sedation. Suitable routes of administration include but are not limited to sublingual, oral, buccal, transdermal, nasal, subcutaneous, intraocular, intravenous, intramuscular and intraperitoneal (e.g., by injection). Preferred routes of administration are oral and transdermal. The therapeutic compounds can be administered with a pharmaceutically acceptable vehicle. Depending on the route of administration, the active compound may be coated in a material to protect the compound from the action of acids, enzymes and other natural conditions which may inactivate the compound.

Therapeutic compounds of the invention can be formulated to ensure proper distribution *in vivo*. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds of the invention cross the BBB, they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, e.g., U.S. Patents 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs ("targeting moieties"), thus providing targeted drug delivery (see, e.g., Ranade *et al.*, 1989). Exemplary targeting moieties include folate or biotin (see, e.g., U.S. Patent 5,416,016 to Low *et al.*); mannosides (Umezawa *et al.*, 1988); antibodies (Bloeman *et al.*, 1995; Owais *et al.*, 1995); surfactant protein A receptor (Briscoe *et al.*, 1995). In a preferred embodiment,

therapeutic compounds of the invention are formulated in liposomes; in a more preferred embodiment, the liposomes include a targeting moiety.

Delivery and *in vivo* distribution can also be affected by alteration of an anionic group of compounds of the invention. For example, anionic groups such as phosphonate or  
5 carboxylate can be esterified to provide compounds with desirable pharmacokinetic, pharmacodynamic, biodistributive, or other properties. Exemplary compounds include IVI and pharmaceutically acceptable salts or esters thereof.

To administer a therapeutic compound by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to  
10 prevent its inactivation. For example, a therapeutic compound may be administered to a subject in an appropriate carrier, for example, liposomes, or a diluent. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes (Strejan *et al.*, *J. Neuroimmunol.* (1984) 7, 27).

15 A therapeutic compound may also be administered parenterally (e.g., intramuscularly, intravenously, intraperitoneally, intraspinally, or intracerebrally). Dispersions can be prepared in glycerol, liquid polyethylene glycols, lactose, dextrose and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms. Pharmaceutical compositions suitable  
20 for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases, the composition must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria  
25 and fungi. The vehicle can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, dextrose, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants.

Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In some cases, it will be preferable to include isotonic agents, for example, sugars, sodium chloride, or polyalcohols such as mannitol and sorbitol, in the composition.

- 5 Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate or gelatin.

10 Sterile injectable solutions can be prepared by incorporating a therapeutic compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filter sterilization. Generally, dispersions are prepared by incorporating the therapeutic compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient (i.e., the therapeutic compound) plus any additional desired ingredient from a previously sterile-filtered solution thereof.

15 A therapeutic compound can be orally administered, for example, with an inert diluent or an assimilable edible carrier. A therapeutic compound and other ingredients may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, a therapeutic compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. The percentage of therapeutic compound in the compositions and preparations may, of course, be varied. The amount of therapeutic compound in such therapeutically useful compositions is such that a suitable dosage will be obtained.

25 It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit containing a predetermined quantity of therapeutic compound calculated to produce the

desired therapeutic effect in association with the required pharmaceutical vehicle. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the therapeutic compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such a therapeutic compound for the treatment of pain and inflammation in subjects, or effecting sedation.

A therapeutic composition can be administered in time-release or depot form, to obtain sustained release of a therapeutic compound over time. A therapeutic compound of the invention can also be administered transdermally (e.g., by providing a therapeutic compound, with a suitable carrier, in patch form, or in an unguent or cream).

Active compounds are administered at a therapeutically effective dosage sufficient to mitigate pain and/or inflammation in a subject. The ability of a compound to mitigate pain or inflammation can be evaluated in model systems that may be predictive of analgesia and anti-inflammation in human diseases, such as animal model systems known in the art (including, e.g., the preclinical acute pain writhing model in the mouse; and the formalin-sensitization model of tissue injury pain in the rat) or by *in vitro* methods, (including, e.g., the assays described above and below, *vide infra*). The ability of a compound to effect sedation can be evaluated in model systems that may be predictive of sedation of use in treatment of human diseases, such as animal model systems known in the art (including, e.g., the loss of the righting reflex in the mouse as described, *vide infra*) or by *in vitro* methods, (including, e.g., modulation of the activity of GABA<sub>A</sub> receptors as described above and below, *vide infra*).

It will be appreciated that the ability of a compound of the invention to mitigate pain and/or inflammation, in certain embodiments, be evaluated by observation of one or more symptoms or signs associated with pain and inflammation *in vivo*. Thus, for example, the ability of a compound to alleviate pain may be associated with an observable improvement in a clinical manifestation of the underlying pain or inflammation related disease state or condition, or a slowing or delay in progression of symptoms of the condition. Thus,

monitoring of clinical manifestations of disease can be useful in evaluating the analgesic, sedative and anti-inflammatory efficacy of a compound of the invention.

Treating or mitigating pain may involve effecting analgesia, effecting sedation, inhibiting or preventing inflammation, and/or ameliorating the manifestations or impact of pain inducing stimuli. Modulating a biological process such as the biological levels of cGMP or cAMP, or activity of soluble GCase, includes regulating increases and decreases in such activity, and inhibition, potentiation, agonism, or antagonism of the biological process.

Methods of the invention are useful for treating pain and/or inflammation associated with any disease in which pain or inflammation occurs. Clinically, pain and inflammation can be associated with, but not limited to, tissue injury, post-operative tissue injury, nerve injury, post-herpetic neuralgia, phantom limb pain, diabetic neuropathy, arthritis, dysmenorrhea, endometriosis, cancer, chemotherapy, myocardial infarction, cerebral vascular occlusion, or result from surgical procedures.

Certain compounds for use in the methods of the invention are commercially available, whereas others are novel (see hereinbelow and applicants' co-pending application USSN 09/267,379, filed March 15, 1999). Both types can be synthesized by standard techniques known in the art. In general, nitrate esters can be prepared from the corresponding alcohol, oxirane or alkene by standard methods that include: nitration of alcohols and oxiranes, mixed aqueous/organic solvents using mixtures of nitric and sulfuric acid and/or their salts, with temperature control (see Yang *et al.*, 1996); nitration of alcohols and oxiranes in acetic anhydride using nitric acid or its salts with or without added acid catalyst, with temperature control (see, e.g., Louw, *et al.*, 1976); nitration of an alcohol with a nitronium salt, e.g., a tetrafluoroborate; nitration of an alkene with thallium nitrate in an appropriate solvent (Ouellette *et al.*, 1976). Compounds of the present invention also can be prepared as described below.

The contents of all scientific publications and patent documents cited herein are hereby incorporated herein by reference in their entirety.

The following Examples further illustrate the present invention and are not intended to be limiting in any respect.

## Examples

### *Example 1: Characterization of guanylyl cyclase activation*

5           Activation of soluble guanylyl cyclase (GCase) by nitrates III<sub>m</sub>, IV<sub>a</sub>, IV<sub>b</sub>, IV<sub>d</sub>, IV<sub>e</sub>, IV<sub>f</sub>, IV<sub>g</sub>, IV<sub>j</sub>, V<sub>a</sub>, V<sub>b</sub>, and GTN was assayed employing partially purified enzyme freshly prepared from the 105,000g supernatant fraction of rat aorta homogenates, using the radioimmunoassay method described by Bennett *et al.* (1992). Dose-response curves were obtained for GCase activation by nitrates IV<sub>a</sub>, IV<sub>b</sub>, IV<sub>d</sub>, IV<sub>e</sub>, IV<sub>f</sub>, IV<sub>g</sub>, IV<sub>j</sub>, and GTN in the  
10           presence and absence of cysteine and dithiothreitol (DTT; both 2mM). In all cases, data were normalized to the maximal GTN response carried out in identical GCase preparations. Experimental incubations were performed at 37°C for 10 min. The data for IV<sub>d</sub> is summarized in Fig. 1. The GCase assay data show that IV<sub>d</sub> activates GCase, with a submillimolar EC-50 (effective concentration for 50% of the subjects) in the absence of any  
15           added thiol, in contrast to GTN, which requires added cysteine. Compounds IV<sub>d</sub> also activates GCase in the presence of DTT, in contrast to GTN, which, enigmatically, does not. Relative to GTN itself, a wide range of potency was observed for these novel nitrate esters. No activation of GCase by glycerol mononitrates was observed in this assay at the concentrations of nitrate employed.

20           Activation of GCase, *in vitro*, by some of these organic nitrates is via release of NO, since in the presence of cysteine, substantial NO is released at rates which are measurable amperometrically, using the method described by Artz and Thatcher (1998). By comparison, nitroglycerin, which is currently understood by others skilled in the art to act only as an NO-donor therapeutic agent, does not release NO at a rate measurable amperometrically, in the  
25           absence nor the presence of cysteine. Relative rates for NO release, at 37°C, pH 7.4, in the presence of cysteine (2mM) from nitrates V<sub>j</sub>, V<sub>u</sub>, V<sub>i</sub>, V<sub>h</sub>, V<sub>g</sub>, V<sub>f</sub>, and V<sub>e</sub>, (1mM) were 1.0, 1.0, 1.8, 0, 1.2, 0.5, 1.8, respectively. Thus modification of the structure of these organic nitrates can be used to control their NO-releasing ability and also to modulate GCase activity.

          To test for potential differences in GCase activation by nitrates, the effects of III<sub>m</sub>,  
30   IV<sub>h</sub>, V<sub>a</sub>, V<sub>b</sub>, and GTN were assayed in brain and vascular tissue. IV<sub>h</sub> had no effect on GCase

activity in either rat aorta or rat hippocampus (Fig. 2). IIIIm had greater efficacy to stimulate GCase activity compared to GTN in both rat aorta and rat hippocampus (Fig. 2). Vb was found to be equivalent to GTN in efficacy and potency for activation of GCase in both rat aorta and rat hippocampus (Fig. 3). Va was found to have greater efficacy, but equal potency, to GTN in rat aorta (Fig. 3a). In contrast, Va had greater efficacy and greater potency to stimulate GCase in rat hippocampus (Fig. 3b). These data illustrate that nitrates have differential effects on GCase activation that are dependent on both structure of the compound and the tissue assayed for GCase activity, supporting the notion that effects of nitrates elicited through GCase activation, such as analgesia and vasodilation, are separable and may be regulated in a tissue-specific and/or activity-specific manner, by appropriate choice of organic nitrate.

As further examples of the potential for modulating potency, efficacy and tissue selectivity for activation of GCase, by choice of an appropriate organic nitrate, nitrates Vaa and Vt were assayed in brain and vascular tissue. In the presence (+) and absence (-) of 1mM cysteine, the potency (EC-50 values) and efficacy (maximal activation) were measured for activation of GCase from rat hippocampus (Table 2).

Table 2	1mM cysteine	EC-50 (hipp.), M	EC-50 (aorta), M	maximal (hipp.), relative to GTN <sup>a</sup>	maximal (aorta), relative to GTN <sup>a</sup>
Vaa	+	$1.4 \times 10^{-4}$	$1.8 \times 10^{-4}$	5.1	2.2
Vaa	-	$5.7 \times 10^{-5}$	$1.8 \times 10^{-4}$	2.1	1.0
Vt	+	$5.1 \times 10^{-5}$	$6.5 \times 10^{-5}$	3.0	2.3
Vt	-	$1.7 \times 10^{-5}$	$1.8 \times 10^{-6}$	1.8	1.7
a) Normalized to GTN + 1mM cysteine maximal response.					

### Example 2: Characterization of cyclic GMP accumulation

In order to extend the GCase data further, the effects of nitrates Va, IIIIm, Vb, Vc, and IVk on cyclic GMP accumulation in intact isolated rat aorta were examined (Figs. 4, 5). Thoracic aortic strips were prepared from male Sprague-Dawley rats (Charles-River, Canada) as described in McGuire *et al.* (1994) and Stewart *et al.* (1989). Tissues were contracted submaximally with phenylephrine (0.1  $\mu$ M) and exposed to various concentrations of drug for 1 min. Cyclic GMP accumulation was determined using the radioimmunoassay method described by Bennett *et al.* (1992). At concentrations of 1  $\mu$ M and 10  $\mu$ M, GTN and IVk significantly increased cGMP accumulation (Figs. 5). At a concentration of 1  $\mu$ M, Va, IIIIm, Vb, and Vc did not significantly increase cyclic GMP accumulation (Figs. 4a, 5a). At a concentration of 10  $\mu$ M, Va, Vb, and IVk significantly increased cyclic GMP accumulation whereas IIIIm and Vc did not (Figs. 4b, 5b).

Sections of rat hippocampus (400  $\mu$ m) were prepared and incubated in oxygenated Krebs solution at 37°C. After a 60-min equilibration period, the brain slices were stimulated with different concentrations of Va or GTN for 3-min. Cyclic GMP accumulation was determined as described above for aortic strips. Figure 6 shows that Va causes a concentration-dependent increase in the tissue levels of cGMP in rat hippocampal brain slices *in vitro*, and that at high concentration (100  $\mu$ M) Va is more effective than GTN in elevating



cGMP levels in hippocampal brain slices *in vitro*. These data are in very good agreement with the differential effects of Va and GTN on hippocampal GCase activity shown in Figure 3b.

*Example 3: Characterization of relaxation of isolated blood vessels*

In order to extend the GCase data, the relaxing effects of nitrates IIIm, IVc, IVd, IVf, IVg, IVh, IVk, Va, Vb, and Vc on rat aortic tissue were examined. Thoracic aortic strips were prepared from male Sprague-Dawley rats (Charles-River, Canada) as described in McGuire *et al.* (1994), and Stewart *et al.* (1989). Tissues were contracted submaximally with phenylephrine (0.1  $\mu$ M) and exposed to various concentrations of nitrovasodilator to obtain concentration-response curves. In this intact tissue assay, all of the nitrates were observed to cause relaxation of the tissue with a maximal relaxant response equal to that obtained with GTN. However, the compounds differed in potency, with EC-50 values of 7.87 nM, 94.3 nM, 6.59  $\mu$ M, 25.2  $\mu$ M, 11.0  $\mu$ M, and 0.203  $\mu$ M, for GTN and compounds Va, IVd, IVg, IVf, and IVc, respectively (Fig. 7,8). In another series of experiments, the EC-50 values for relaxation were 0.61 nM, 3.19 nM, 8.40 nM, 0.153  $\mu$ M, 0.437  $\mu$ M and 6.89  $\mu$ M for GTN, IVk, Vb, IIIm, Vc, and IVh, respectively (Fig. 7,8). Compounds IVd and IVc were tested for their ability to cause vascular relaxation in tissues that had been made tolerant to the relaxant effect of GTN. GTN tolerance was induced by incubating tissues with high concentrations of GTN (0.5mM GTN for 30 min). Under these conditions, the maximal relaxant effects of IVd (Fig. 12a) and IVc (Fig. 12b) were not significantly different to that of untreated tissue. The EC-50 for relaxation was increased approximately threefold, but the difference was not statistically significant.

*Example 4: Characterization of blood pressure changes in the whole animal*

To test for differential effects of nitrates on blood pressure responses, Va and GTN were injected into rats in which the abdominal aorta was cannulated for blood pressure recording. In the first experiment, Va and GTN were injected subcutaneously at a dose of 400  $\mu$ mol/kg body weight into conscious, freely moving animals. GTN caused a small and transient decrease in blood pressure in these animals, whereas Va had no discernable effect on

arterial blood pressure (Fig. 9). Va and GTN were subsequently tested in anesthetized rats in which the abdominal vena cava was also cannulated to allow for bolus intravenous injection of drugs. In this preparation, GTN caused a substantial and dose-dependent decrease in arterial blood pressure. In contrast, Va at equal doses had very modest effects on blood pressure at  
 5 doses lower than 2  $\mu\text{mol/kg}$  body weight (Fig. 10). These data are in very good agreement with the results obtained for these two agents using the isolated blood vessel preparation.

The plasma levels of nitrates Vb and Vc (the denitrated metabolite of Vb) were measured to gain insight into the handling of these molecules in the body. Cannulas were placed in the abdominal aorta for blood sampling. After a two-day recovery period, a single  
 10 subcutaneous dose of Vb (200  $\mu\text{mol/kg}$ ) was administered and blood samples collected over a period of six hours. Samples were centrifuged, the plasma collected, and the concentration of Vb and Vc determined by gas-liquid chromatography by the method of McDonald and Bennett (1990). The data obtained for Vb and Vc indicate that nitrates achieve maximal plasma levels within 30 minutes after subcutaneous injection, and thereafter decline at a steady  
 15 rate (Fig. 11). These data suggest that nitrates have excellent bioavailability after subcutaneous injection.

*Example 5: Characterization of the analgesic effects of novel organic nitrates in a model of acute pain*

20 Injection of dilute acetic solutions into the peritoneum of a mouse induces writhing movements that can be quantified. We adopted the methodology described by Bak *et al.* (1998) to test for analgesic effects of organic nitrates in this mouse model. Each mouse was given an intraperitoneal injection of 0.5 mL of a 0.6% solution of acetic acid in distilled water. After a 5 minute delay, the number of writhing movements was counted over a 10 minute  
 25 period. To test the efficacy of novel organic nitrates in this model, drugs were administered at doses of 100-500 mg/kg (given by subcutaneous injection) 15 minutes before the intraperitoneal injection of acetic acid. In this model of acute pain, Vm induced a significant, dose-dependent analgesic effect, manifested as a decrease in the number of writhes per 10 minute period after intraperitoneal injection of dilute acetic acid (Figure 13a). Va was also

able to act as an analgesic (decreased writhing) in this experimental model when administered at a dose of 500 mg/kg subcutaneously (Figure 13b).

*Example 6: Characterization of the analgesic effects of novel organic nitrates in a model of hyperalgesia/allodynia*

Under light halothane anesthesia, male Sprague-Dawley rats were injected subcutaneously with 0.05 mL of 5% formalin into the dorsal surface of one hind paw as described in Malmberg and Yaksh (*Anesthesiology* (1993) 79, 270-281). The number of spontaneous paw flinches was determined in 1 minute blocks at 5 minute intervals for 60 minutes. Formalin injection in the paw produces two distinct phases of pain; an acute phase occurring within the first 5-10 minutes, and a delayed phase that develops between 15-30 minutes after formalin injection. The acute phase of the pain response to formalin is caused by activation of peripheral nociceptive sensory afferents (C-fibres) by the peripheral stimulus. The delayed pain response is considered to be a hyperalgesia/allodynia caused by a combination of sensitization of peripheral sensory afferents and sensitization of synaptic connections in the spinal cord. The formalin test in the rat is considered to be an appropriate model for tissue injury pain occurring in humans (Tjolsen *et al.*, *Pain* (1992) 51, 5-17; Yaksh, *TIPS* (1999) 20, 329-337). In this experimental model of inflammatory tissue injury pain, Vm (500 mg/kg) significantly reduced both phases of the pain response to formalin injection (Fig. 14a,b).

*Example 7: Synthesis of IIIe*

To acetic anhydride (3 mL) was added gradually, with stirring, 70% nitric acid (0.26 mL), while keeping the temperature between 20-30° by external cooling. With continuous vigorous stirring the mixture was cooled to -30-35° and 2',3'-dideoxy-3-thiocytosine (0.25 g) was added. After 10 min at -35°, the reaction mixture was heated up to -20° and then stirred at -20-10° for 15 min and 10 min at 0°. The resulting reaction mixture was poured into ice-water, stirred for 1 h, then NaHCO<sub>3</sub> was added by portions until CO<sub>2</sub> evolution ceased. The water solution was extracted with 3x20 mL of ethyl acetate. Combined extracts were dried

(MgSO<sub>4</sub>) and concentrated. 0.38 g of slightly yellowish oil was obtained. The oil crystallized in a day and was recrystallized from CHCl<sub>3</sub>. Yield 52%. Conversion to the nitrate was evidenced by the significant downfield shift of the C5' proton multiplet from  $\delta$  3.6 to 4.85 ppm.

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*Example 8: Synthesis of nitrate IIIf*

0.26 mL (4.15 mmol) conc. HNO<sub>3</sub> was added to 2 mL acetic anhydride such that the temperature did not exceed 25 – 30 °C. The mixture was cooled at 0 – 5 °C and 0.3 g (1.88 mmol) of 5-(1,2-dihydroxyethyl)-4-methylthiazole was added in several portions, the temperature being kept below 5 °C. The reaction mixture was stirred at 0 – 5 °C for 45 min and then 0.45 mL water was added. The mixture was stirred for 30 min and then rotavary evaporated. The residue was neutralized by adding 5 mL of saturated NaHCO<sub>3</sub> solution and the organic product was extracted with ethyl acetate. The organic layer was concentrated and the dinitrate IIIf was purified through column chromatography (silica gel/ ethyl acetate eluant). A slightly yellow solid was obtained. Yield: 0.150 g (32 %).

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*Example 9: Synthesis of nitrate IIIi*

Nitrate IIIi was obtained by two routes. Route I proceeded from the elimination reaction of IIIIm in basic solution. Route II proceeded from nitration of *trans*-3-bromo-4-hydroxytetrahydrothiophene-1,1-dioxide, yielding nitrate IIIIn, followed by reaction with a weak base, e.g., sodium thiocyanate in 2-butanone. Purification may be achieved with silica flash column chromatography using 1:1 hexane:ethyl acetate as eluant.

20

*Example 10: Synthesis of nitrate IIIj*

1,4-Dibromo-2,3-butanediol may be nitrated: (a) using a nitration mixture prepared from HNO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub> over 2 days; or (b) using acetyl nitrate reacting for 2 hours. Work-up requires quenching of the reaction mixture in ice-water for an hour, extraction, drying, and evaporation. Successful purification of the title compound by silica gel column

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chromatography is achieved on a 25 g scale using a mixture of 70% hexane and 30%  $\text{CH}_2\text{Cl}_2$  as eluent.

*Example 11: Synthesis of nitrate Ve*

5 4-Methylbenzenethiol was obtained by adaptation of literature procedures from p-toluidine (J.-P. Morizur, *Bull. Soc. Chim. Fr.* (1964) 1338-1342; Bourgeois, *Recl. Trav. Chim. Pays-Bas* (1899) 18, 445-450). p-Toluidine hydrochloride (14.2 g, 0.098 mole) was diazotised at 5°C with concentrated hydrochloric acid (16.5 mL) and sodium nitrite (7.2 g, 0.104 mole) in water (12 mL). The solution of diazonium salt was added over 1.5 h to a solution of ethyl  
10 xanthate (24 g, 0.149 mole) in water (30 mL) at 45-50°C. The mixture was kept at this temperature, under stirring, for a further 1 h. The xanthate ester was separated as a maroon oil, washed with 50 mL 10 % NaOH and with water to neutral pH and dried over  $\text{MgSO}_4$  (20g of crude product). The crude xanthate was dissolved in 60 mL absolute ethanol and to this solution 20 g KOH (pellets) were added in portions. The reaction mixture was refluxed  
15 under stirring and Ar for 8 h, then concentrated under vacuum. The concentrate was taken up in 50 mL  $\text{H}_2\text{O}$  and extracted with 3x100 mL diethyl ether. The aqueous layer was acidified with a 6N  $\text{H}_2\text{SO}_4$  solution and extracted with 3x100 mL  $\text{CH}_2\text{Cl}_2$ . The combined extracts were washed with water, dried over  $\text{MgSO}_4$ , evaporated and flash columned on silica gel, eluant hexanes:ethyl acetate=9:1, giving 10 g (81.56 %) of 4-methylbenzenethiol.  $^1\text{H}$ -  
20 NMR( $\text{CDCl}_3$ , 300MHz):7.18-7.24 (m, arom 2H), 7.04-7.11 (d, arom 2H, J 7.93), 3.41(s, 1H), 2.32(s, 3H).  $^{13}\text{C}$ -NMR(75.48 MHz): 21.34, 128.95, 130.24, 130.29, 136.05.

The dinitrate IVd (9.67 mmoles) was dissolved in 10 mL distilled water and the solution kept under Ar for 30 minutes. To this solution, a solution of 0.8 g (6.46 mmoles) of 4-methylbenzenethiol and 7 mL 1M NaOH was added dropwise. The resulting emulsion was  
25 stirred for 15 min and then extracted with 3x20 mL  $\text{CH}_2\text{Cl}_2$ . The combined organic extracts were washed with  $\text{H}_2\text{O}$ , dried over  $\text{MgSO}_4$  and concentrated under vacuum. The remaining oil was purified by flash column chromatography on silica gel, eluant hexanes: ethyl acetate=9:1, giving the product Ve (1.097 g, 52.22 %).  $^1\text{H}$ -NMR( $\text{CDCl}_3$ , 300MHz):7.44-7.51 (m, arom 2H), 7.17-7.24 (d, arom 2H, J 7.91), 5.47-5.59 (m, 1H), 4.83-4.93 (dd, 1H, J 12.81,

2.78), 4.57-4.67 (dd, 1H, J 12.82, 5.71), 3.02-3.12 (dd, 1H, J 14.48, 6.01), 2.9-2.99 (dd, 1H, J 14.47, 7.72), 2.38 (s, 3H).  $^{13}\text{C}$ -NMR(75.48 MHz): 21.53, 36.78, 69.82, 77.68, 130.52, 130.62, 132.55, 139.23.

#### 5 *Example 12: Synthesis of nitrate IIIm*

3,4-Epoxytetrahydrothiophene-1,1-dioxide (250 mg, 1.9 mmol) was refluxed for 24 h in 10 mL of water and 25 mg of toluenesulfonic acid. After the first 6 h, another 25 mg of the acid was added. The reaction was monitored by thin layer chromatography (TLC) (5% methanol in dichloromethane). Purification was by Si flash column chromatography using 10 5% methanol/ $\text{CH}_2\text{Cl}_2$  as eluent to afford 200 mg of diol. The diol was nitrated in a cooled solution of conc. sulfuric acid (2 mol eq.), nitric acid (70%, 2 mol eq.) in an ice bath. The temperature was maintained as close to  $0^\circ\text{C}$  as possible. The ice bath was removed and the mixture was allowed to stir for 1 hour (reaction was monitored by TLC, 100%  $\text{CH}_2\text{Cl}_2$  eluent). The acid layer was removed and the organic layer washed with: (i) water; (ii) 10% sodium carbonate; (iii) 10% urea; (iv) water. Drying over sodium sulfate, filtration and 15 concentration, yielded crude product which was purified by flash column chromatography, with dichloromethane as eluent. An alternative route involves direct nitration of 3,4-epoxytetrahydrothiophene-1,1-dioxide in a similar nitration mixture.

#### 20 *Example 13: Synthesis of nitrate IVk*

1.17 mL (18.2 mmol) concentrated  $\text{HNO}_3$  was added, under stirring and cooling ( $0 - 5^\circ\text{C}$ ), to 1 mL (18.2 mmol) concentrated  $\text{H}_2\text{SO}_4$  and then 2 g (14 mmol) of 4-methyl-5-(2-hydroxyethyl)thiazole was added dropwise into the nitration mixture, the temperature being kept under  $10^\circ\text{C}$ . The mixture was stirred for 3 hours at room temperature, diluted with 10 25 mL of water and neutralized with solid  $\text{NaHCO}_3$ . The organic product was extracted with ethyl acetate and purified by column chromatography (silica gel/ ethyl acetate eluant) to produce a colorless oily product. Yield: 1.18 g (45%).

*Example 14: Synthesis of nitrate IVi*

0.03 g (0.035 mL) of allyl cyanide was added to a stirred suspension of 0.22 g (0.5 mmol) of  $\text{Tl}(\text{NO}_3)_3 \cdot 3\text{H}_2\text{O}$  in 2 mL of pentane. After 20 min of vigorous stirring the pentane solution was decanted and evaporated to dryness. After evaporation the residual oil (0.44 g) was columned ( $\text{CH}_2\text{Cl}_2$ ,  $R_f$  0.64 ( $\text{CH}_2\text{Cl}_2$ )). Clean oil immediately crystallized during attempt to dissolve it in  $\text{CDCl}_3$ . Yield 0.065 g (76%). The structure of IVn was confirmed by X-ray analysis. IR (film): 1297.03, 1678.91, 2258.91 (CN). Mass spec.  $m/z$  ( $\text{CI}^+$  fragment, %): 191.9 ( $\text{M} + \text{H}$ , 2.44), 129.0 (16.41), 81.9 (100). Calculated for  $\text{C}_4\text{H}_5\text{N}_3\text{O}_6$  191.02.

*Example 15: Synthesis of nitrate IVm*

0.9 g (0.75 mL, 4.92 mmol) of allylphenyl sulfone was added dropwise to a stirred suspension of 2.43 g (5.47 mmol) of  $\text{Tl}(\text{NO}_3)_3 \cdot 3\text{H}_2\text{O}$  in 10 mL of pentane. The resulting mixture was stirred overnight. The pentane solution was decanted. 2x10 mL of methanol were added to the reaction mixture, stirred for 10 minutes and extracts were added to the pentane solution. The combined extracts evaporated to dryness and purified by silica flash column chromatography using  $\text{CH}_2\text{Cl}_2$  as eluant. Yield 0.08 g (15%). IR (KBr): 1152.39, 1290.91, 1273.12, 1353.83, 1646.08. Mass spec.  $m/z$  ( $\text{CI}^+$  fragment, %): 307.0 ( $\text{M} + 1$ , 66.5), 244.0 (100%). Calculated for  $\text{C}_9\text{H}_{10}\text{N}_2\text{O}_8\text{S}$  306.02.

*Example 16: Synthesis of nitrate Va*

2.2 g (7.3 mmol) of nitrate IVd was dissolved in 5 g of cold  $\text{H}_2\text{O}_2$  (30%, 0°C) and then 1 g of 10 %  $\text{H}_2\text{SO}_4$  was added. The mixture was stirred at 0-5 °C until a white oil separated (ca. 30-60 min). The aqueous layer was discarded and the oil was dissolved in dichloromethane, washed successively with water, then  $\text{NaHCO}_3$  solution and finally water. The organic solution was dried over  $\text{MgSO}_4$ . Removal of the solvent produced 1.3 g of the crude product which was purified by column chromatography (Silicagel,  $\text{CH}_2\text{Cl}_2$ /hexanes : 70/30). Yield: 0.650 g (45 %).

*Example 17: Synthesis of nitrate Vc*

3 g (8.88 mmol) of 1,4-dibromo-2,3-dinitrobutanediol and 2.81 g (18 mmol) of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  were dissolved in the mixture of 100 mL of methanol and 45 mL of  $\text{H}_2\text{O}$ . The resulting solution was heated during 4 days at 40-45°. After this time the reaction mixture was partially evaporated to reduce the volume of solvents. The resulting mixture was extracted 4x50 mL of ethyl ether. The extracts were combined, washed ( $\text{H}_2\text{O}$ ), dried ( $\text{MgSO}_4$ ) and evaporated to minimum. Column chromatography afforded the title compound in 10% yield, separated from Vb the major product.

*Example 18: Synthesis of nitrate Vy*

The title compound was synthesised by the reaction of IVd with 4-methyl-5-thiazole ethanthiol, in a similar procedure to that used in Example 11. The crude product was purified by column chromatography (Silicagel, ethyl acetate eluant) to give product (50 mg, 27.32 %).  $^1\text{H-NMR}(\text{CDCl}_3, 300\text{MHz})$ : 8.22 (s, 1H), 5.49-5.6 (m, 1H), 4.9-5.00 (dd, 1H), 4.64-4.78 (dd, 1H), 3.14-3.22 (t, 2H), 2.89-3.07 (m, 4H), 2.45 (s, 3H). Mass spec.,  $m/z$  ( $\text{EI}^+$ , fragment, %): 355.0. Calculated for  $\text{C}_9\text{H}_{13}\text{N}_3\text{O}_6\text{S}_3$ : 355.0. The 4-Methyl-5-thiazole precursor was obtained from 4-methyl-5-thiazole ethanol, thiourea and hydrobromic acid by adaptation of literature procedures (R.L. Frank, P. V. Smith, *J. Am. Chem. Soc.* (1946) 68, 2103-2104). A mixture of 2 g 4-methyl-5-thiazole ethanol (13.965 mmoles), 1.063 g (13.965 mmoles) thiourea and 9.45 g (56 mmoles) hydrogen bromide as 48 % hydrobromic acid was refluxed for 7 h with stirring, under Ar. A solution of 2.24 g (56 mmoles) of NaOH in 20 mL of water was then added and the mixture was refluxed without stirring for 2 h. The layers were separated, and the acidified aqueous layer was extracted with three 30 mL portions of  $\text{CH}_2\text{Cl}_2$ . The extracts and original organic layer were combined, dried over  $\text{MgSO}_4$  and concentrated under vacuum to afford 1.9 g crude product which was purified by column chromatography using ethyl acetate as eluent (Yield 1.6 g, 75%).  $^1\text{H-NMR}(\text{CDCl}_3, 300\text{MHz})$ : 8.5 (s, 1H), 2.94-3.02 (t, 2H), 2.62-2.72 (q, 2H), 2.33 (s, 3H), 1.38-1.45 (t, 1H).  $^{13}\text{C-NMR}(75.48 \text{ MHz})$ : 15.41, 26.41, 31.51, 39.72, 129.36, 149.90



*Example 19: Synthesis of nitrate IIIk and IIIl*

Synthesis from dinitrate IIIj proceeded by refluxing with sodium or potassium thiocyanate (2 eq.) in 2-butanone for 8 h. After cooling, a precipitate was removed by filtration and the filtrate was concentrated. Nitrates IIIk and IIIl were separated by silica flash column chromatography with hexane/dichloromethane as eluent.

*Example 20: Synthesis of nitrate V<sub>k</sub>.*

IVd (0.43 g, 1.37 mmol) was dissolved in 10 mL of distilled water and the emulsion of 0.23 g (1.28 mmol) of the ethyl ester of thiosalicylic acid in 1.3 mL of 1 M NaOH was added. The resulting solution immediately became turbid and was stirred for 3 min, then extracted with ethyl acetate (4x15 mL). The resulting extracts were washed with H<sub>2</sub>O, dried (MgSO<sub>4</sub>), and concentrated by evaporation. The residue was flash columned on silica gel, eluant hexane:ethyl acetate = 9:1 (R<sub>f</sub> 0.23), giving 0.27 g (55%) of V<sub>k</sub>. <sup>1</sup>H-NMR(CDCl<sub>3</sub>, 300 MHz): 8.06-8.12 (1H, dd, J 8.12, 0.36), 7.99-8.04 (1H, dd, J 7.8, 1.15), 7.51-7.59 (1H, m, J 7.24, 1.44), 7.21-7.29 (1H, m, J 7.35, 0.54), 5.40-5.49 (1H, m), 4.70-4.78 (1H, dd, J 13.04, 2.95), 4.33-4.45 (3H, m, superposition of dd (J 13.08, 6.01) of 1H from CH<sub>2</sub>-ONO<sub>2</sub> and quart. from O-CH<sub>2</sub>-CH<sub>3</sub>), 2.66-2.87 (2H, m), 2.06-2.15 (2H, quart., J 6.92), 1.35-1.43 (3H, t, J 7.14). <sup>13</sup>C-NMR: (CDCl<sub>3</sub>, 75.48 MHz): 166.21, 140.41, 132.78, 131.46, 127.63, 125.48, 77.25, 71.03, 61.42, 32.51, 28.29, 14.17. Mass spec. m/z (EI<sup>+</sup>, fragment, %): 392.3 (M<sup>+</sup>, 3.69), 153 (100). Calculated for C<sub>13</sub>H<sub>16</sub>N<sub>2</sub>O<sub>8</sub>S<sub>2</sub> 392.03.

*Example 21: Chiral synthesis of nitrate IVd*

A 50 mL round-bottom flask, equipped with a magnetic stirrer, was charged with 10 mL of *tert*-butyl alcohol, 10 mL of water, and 2.8 g of a catalyst (AD-mix-β, Aldrich: K.B.Sharpless, W.Amberg, Y.L.Bennani, G.A.Crispino, J.Hartung, K.-S.Jeong, H.-L.Kwong, K.Morikawa, Z.-M.Wang, D.Xu, X.-L.Zhang, *J.Org.Chem.* (1992) 57, 2768-2771). Stirring at room temperature produced two clear phases; the lower aqueous phase appears bright yellow. The mixture was cooled to 4° and 0.2 mL (2 mmol) of allylbromide was added at once, and the heterogeneous slurry was stirred vigorously at 4-5° for 2.5 h (monitoring by TLC

hexane:methanol=1:9). While the mixture was stirred at 0°C, solid sodium sulfite (3 g) was added and the mixture was allowed to warm to room temperature and stirred for 1 h. Then 20 mL of ethyl acetate was added to the reaction mixture, and after separation of the layers, the aqueous phase was further extracted with ethyl acetate. The combined organic extracts were dried (MgSO<sub>4</sub>), concentrated *in vacuo* and purified by flash chromatography on silica (hexane:methanol=1:9, R<sub>f</sub> 0.5), to yield chiral 1-bromo-2,3-propanediol. Yield 0.2 g (55.5%). Optical rotation: minus. Nitration to 1-bromo-2,3-dinitroxypropane was achieved using reaction in HNO<sub>3</sub>/H<sub>2</sub>SO<sub>4</sub> (K. Yang, J.D. Artz, J. Lock, C. Sanchez, B.M. Bennett, A.B. Fraser, G.R.J. Thatcher, *J.Chem.Soc., Perkin Trans.* (1996) 1, 1073-1075) and the product was purified by flash chromatography on silica gel (hexane:CH<sub>2</sub>Cl<sub>2</sub> =2:3, R<sub>f</sub> 0.5). Yield 55.5%. Optical rotation: minus. The dinitrate *IVd* was synthesised from chiral 1-bromo-2,3-propanediol by our usual procedure (K. Yang, J.D. Artz, J. Lock, C. Sanchez, B.M. Bennett, A.B. Fraser, G.R.J. Thatcher, *J.Chem.Soc., Perkin Trans.* (1996) 1, 1073-1075). and purified by flash chromatography on silica (ethyl acetate:methanol=9:1). Yield 2.27%. Optical rotation: plus.

#### Example 22: Chiral synthesis of nitrate *Vk*

The same procedure as described above for racemic *Vk* was utilized for the stereospecific synthesis of *Vk* from the stereochemically resolved, chiral dinitrate *IVd*.

#### Example 23: Synthesis of tetranitrate *Vx*

The title compound was synthesised by the reaction of *IVd* (0.345g, 1.15 mmol) with captopril methyl ester (0.2 g, 0.865 mmol) in the presence of 1 mL of 1M NaOH. The crude product was purified by column chromatography (Silicagel, ethyl acetate) to afford 0.1 g (18.03 %) of unsymmetrical disulphide. <sup>1</sup>H-NMR(CDCl<sub>3</sub>, 300MHz): 5.49-5.61 (m, 1H), 4.88-4.90 (m, 1H), 4.62-4.74 (m, 1H), 4.40-4.55 (m, 1H), 3.59-3.78 (m, superpos. 5H), 2.69-3.21 (m, superpos. 5H), 2.14-2.29 (m, 1H), 1.90-2.12 (m, 3H), 1.22-1.28 (d, 3H). <sup>13</sup>C-NMR(75.48 MHz): a mixture of enantiomers can be seen: 17.39, 25.20, 29.40, 36.41, 36.51, 38.55, 41.94, 42.19, 47.32, 52.56, 59.12, 69.90, 70.29, 77.89, 78.00, 173.01, 173.48.

*Example 24: Synthesis of nitrate Vq*

The title compound was synthesised by the reaction of Bunte salt (2.4 g, 8 mmol) with 4,4'-thiobisbenzenethiol (0.5 g, 2 mmol) in the presence of 4.4 mL of 1M NaOH. The crude product was purified by column chromatography (silica gel, hexanes/CH<sub>2</sub>Cl<sub>2</sub>: 3/7) to afford 0.28 g (21.82 %) disulphide. <sup>1</sup>H-NMR(CDCl<sub>3</sub>, 300MHz): 7.2-7.6 (m, arom 8H), 5.42-5.56 (m, 2H), 4.82-4.95 (dd, 2H), 4.55- 4.67 (dd, 2H), 2.93-3.15 (m, 4H).

*Example 25: Synthesis of nitrate Vr*

The title compound was synthesised by the reaction of IVd (0.5 g, 1.58 mmol) with ethyl 2-mercapto-3-(3',4'-methylenedioxy-phenyl) propenoate (0.2 g, 0.8 mmol). The crude product was purified by column chromatography (Silicagel, hexanes/CH<sub>2</sub>Cl<sub>2</sub>: 3/7) to give Vr (0.1 g, 28.09 %). <sup>1</sup>H-NMR(CDCl<sub>3</sub>, 300MHz): 7.97 (s, 1H), 7.48-7.51 (d, 1H), 7.17-7.22 (m, 1H), 6.84-6.89 (d, 1H), 6.05 (s, 2H), 5.48-5.58 (m, 1H), 4.81-4.89 (dd, 1H), 4.53-4.61 (dd, 1H), 4.25-4.4 (m, 2H), 3.05-3.10 (m, 2H), 1.35-1.45 (t, 3H). <sup>13</sup>C-NMR(75.48 MHz): 14.17, 36.27, 62.15, 69.71, 76.57, 101.77, 108.27, 110.31, 125.20, 127.55, 127.87, 146.87, 147.81, 149.76, 165.89.

*Ethyl 2-mercapto-3-(3',4'-methylenedioxy-phenyl) propenoate* was obtained by the following route:

(1) Ethylisothiocyanate (10 g, 0.115 moles), 7.9 g (0.086 moles) mercaptoacetic acid and 5 mL pyridine in C<sub>6</sub>H<sub>6</sub> was refluxed, cooled and filtered yielding 3-ethylrhodanine, which was used in the next step without any further purification. Reaction of 3-ethylrhodanine with piperonal in the presence of sodium acetate was performed in CH<sub>3</sub>OH under refluxing for 1 h. The yellow precipitate obtained after cooling and filtration, was washed several times with CH<sub>3</sub>OH on the filter and crystallized from CH<sub>3</sub>OH to give 3-ethyl-5-piperilidenerodanine. <sup>1</sup>H-NMR(DMSO-d<sub>6</sub>, 300MHz): 7.72 (s, 1H), 7.1-7.3 (m, 3H), 6.14 (s, 2H), 3.95-4.15 (q, 2H), 1.15-1.20 (t, 3H). <sup>13</sup>C-NMR(75.48 MHz): 12.76, 40.28, 103.07, 110.21, 110.47, 120.68, 127.89, 128.07, 134.01, 149.23, 150.74, 167.57, 193.68.

(2) 3-Ethyl-5-piperilidene-rodanine (1g, 3.4 mmol) was added to a stirred solution of 0.16 g (6.8 mmol) in 8 mL abs. ethanol and the mixture was refluxed for 30 min. To the solution cooled to room temperature 5 mL H<sub>2</sub>O were added and the mixture was hydrolyzed

with 10% HCl and extracted with ether. The ether phase was separated, dried ( $\text{MgSO}_4$ ) and evaporated to an oil. Since the investigation by TLC of the crude reaction mixture indicated the presence of piperonal, the reaction mixture was dissolved in  $\text{CH}_2\text{Cl}_2$  and the obtained solution was washed with 1M NaOH. The aqueous phase was extracted twice with  $\text{CH}_2\text{Cl}_2$  and then neutralized with dilute HCl. Free thiol was extracted with ethyl ether. After concentration, the product was purified by column chromatography eluting with hexane/ethyl ether:8/2. Yield 0.4 g (62.4 %).  $^1\text{H-NMR}(\text{CDCl}_3, 300\text{MHz})$ : 7.69 (s, 1H), 7.7.25-7.29 (d, 1H, J 1.46), 7.1-7.18 (m, 1H), 6.85-6.9 (d, 1H, J 8.13), 6.01 (s, 2H), 4.75 (s, 1H), 4.28-4.38 (q, 2H, J 7.12), 1.35- 1.42 (t, 3H, J 7.13).  $^{13}\text{C-NMR}(75.48 \text{ MHz})$ : 14.20, 62.5, 101.41, 108.37, 109.43, 121.06, 125.45, 129.20, 134.68, 147.80, 148.05, 165.43.

#### Example 26: Synthesis of nitrate IVs

The title compound was synthesised by the reaction of IVb with dithiothreitol in  $\text{CH}_3\text{OH}$  and was isolated as an oil in 15-20% yield (CAUTION: stench).  $^1\text{H-NMR}(\text{CDCl}_3, 300 \text{ MHz})$ : 5.23-5.32 (1H, m), 4.87 (1H, dd, J 12.82, 3.22), 4.68 (1H, dd, J 12.83, 6.09), 2.77-2.94 (2H, m), 1.66 (1H, t, J 9.07).  $^{13}\text{C-NMR}$ : ( $\text{CDCl}_3$ , 75.48 MHz): 79.39, 69.30, 23.68.

#### Example 27: Synthesis of nitrate IVt

The title compound was synthesised by the reaction of nitrate IVs with acetyl chloride in  $\text{CHCl}_3$ . Isolated yield 50%.  $^1\text{H-NMR}(\text{CDCl}_3, 300 \text{ MHz})$ : 5.29-5.38 (1H, m), 4.76 (1H, dd, J 12.94, 3.11), 4.55 (1H, dd, J 12.94, 6.37), 3.30 (1H, dd, J 14.06, 5.98), 3.13 (1H, dd, J 14.61, 6.35).  $^{13}\text{C-NMR}$ : ( $\text{CDCl}_3$ , 75.48 MHz): 194.10, 77.00, 69.79, 30.42, 27.78. Mass spec. m/z ( $\text{EI}^+$ , fragment, %): 240.0 ( $\text{M}^+$ , 1.17), 193.9 ( $\text{M-NO}_2$ , 10.86), 148.8 (100). Calculated for  $\text{C}_5\text{H}_8\text{N}_2\text{O}_7\text{S}$  240.01.

#### Example 28: Synthesis of nitrate IIIw

Diethyl 1-chloro-2-trimethylsiloxypentylphosphonate was obtained by adaptation of literature methods (T.Azuhata, Y.Okamoto, *Synthesis* (1983) 916-917). This phosphonate was quantitatively converted to diethyl 1-chloro-2-hydroxypentylphosphonate using  $\text{CH}_3\text{OH}$ .

After stirring for 15 min the resulting reaction mixture was evaporated to a minimum and subjected to nitration with a mixture of  $\text{HNO}_3$  and  $\text{H}_2\text{SO}_4$ . Work-up and flash column chromatography on silica (ethyl acetate eluant) yielded pure product in 25% yield.  $^{31}\text{P}$  ( $\text{CDCl}_3$ , 162 MHz): 24.60.  $^1\text{H}$  ( $\text{CDCl}_3$ , 300 MHz): 5.31-5.45 (m, 1H), 3.92-4.08 (m, 4H), 3.63-3.81 (m, 2H), 2.03-2.30 (m, 2H), 1.16-1.24 (superposition of 2 t, 6H, J 7).  $^{13}\text{C}$  ( $\text{CDCl}_3$ , 75 MHz): 76.83, 62.15 (d, J 6.37), 43.77 (d, J 8.95), 27.08 (d, J 142.00), 15.99 (d, J 5.88).

#### Example 29: Synthesis of nitrate IIIx

Treatment of IIIw with 1 mole of  $\text{Me}_3\text{SiBr}$  for 1 h with subsequent addition of  $\text{CH}_3\text{OH}$  provided the monodealkylated phosphonic acid in high purity. Transformation of the free acid to its sodium salt IIIx was achieved using the cation-exchange resin Amberlite IR-122- $\text{Na}^+$  form.  $^{31}\text{P}$  ( $\text{CD}_3\text{OD}$ , 122 MHz): 17.62.  $^1\text{H}$  ( $\text{CD}_3\text{OD}$ , 300 MHz): 5.38-5.63 (m, 1H), 3.75-4.25 (superposition of 2 m, 4H), 1.88-2.20 (m, 2H), 1.12-1.28 (t, 3H).  $^{13}\text{C}$  ( $\text{CD}_3\text{OD}$ , 75 MHz): 81.14, 61.17 (d, J 5.41), 45.56 (d, J 5.94), 29.35 (d, J 131.74), 17.00 (d, J 6.75)

#### Example 30:

The effect of IVk was tested in *Xenopus* oocytes expressing human recombinant  $\text{GABA}_A$  receptors by the two-electrode voltage clamp technique as described in Reynolds and Maitra (*European Journal of Pharmacology* (1996) 314, 151-156.). The control GABA response was substantially potentiated by the positive control drug, chlormethiazole, at a concentration of 200  $\mu\text{M}$  (Figure 15a). In contrast, a concentration of 200  $\mu\text{M}$  of IVk produced a smaller, but still significant, potentiation of the control GABA response in the same oocyte (Figure 15a). A decreased efficacy with IVk suggests the possibility that it is a partial allosteric modulator of  $\text{GABA}_A$  receptor function, and may therefore have more selective behavioural effects than more efficacious compounds.

The effect of a drug that induces brief periods of sedation/hypnosis in animals is characterized by loss of the righting reflex (loss of the ability of the animal to right its posture when placed on its back). The "loss of the righting reflex" response is a commonly used test for sedative-hypnotic agents. IVk produced a dose-dependent and reversible loss of the

righting reflex in mice when given by intraperitoneal injection at doses of 100 and 200 mg/kg (Figure 15b). Thus, the organic nitrate IVk exhibits the activity of a sedative/hypnotic, and represents a novel class of compounds that may have utility as new anti-anxiety, sedative and/or hypnotic agents.

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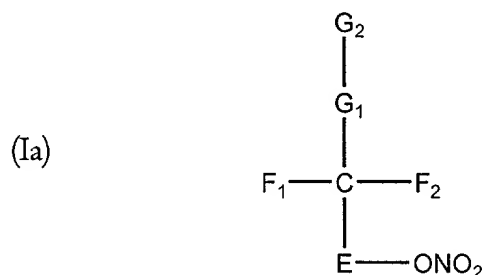
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What is claimed is:

1. A method for treating pain, treating inflammation or providing analgesia in a subject, comprising administering to a subject in need thereof an effective amount of a therapeutic compound, wherein the therapeutic compound is of the formula (Ia):



in which E, F<sub>1</sub>, F<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub> are the same or different organic radicals which may be joined in cyclic ring systems, and which may contain inorganic counterions;

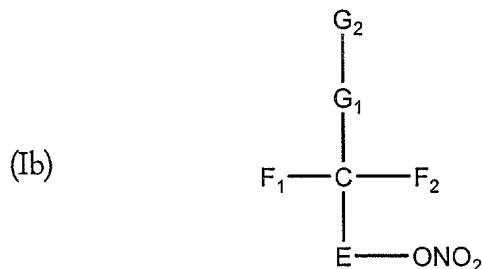
with the proviso that when E and G<sub>1</sub> are methylene groups and F<sub>1</sub> is H, G<sub>2</sub> is not a nitrate group, nor R<sup>N</sup>-Z<sup>N</sup>;

wherein R<sup>N</sup> is any aryl or heteroaryl group and Z<sup>N</sup> is (CO)<sub>mm</sub>-X<sup>N</sup><sub>nn</sub>-Y<sup>N</sup><sub>oo</sub>;

wherein mm, nn, oo are 0 or 1 and X<sup>N</sup>, Y<sup>N</sup> are NH, NR<sup>NN</sup>, O or CH<sub>2</sub>;

wherein R<sup>NN</sup> is a short chain alkyl group (C<sub>1</sub> - C<sub>12</sub>).

2. A method for treating pain, treating inflammation or providing analgesia in a subject, comprising administering to a subject in need thereof an effective amount of a therapeutic compound, wherein the therapeutic compound is of the formula (Ib):



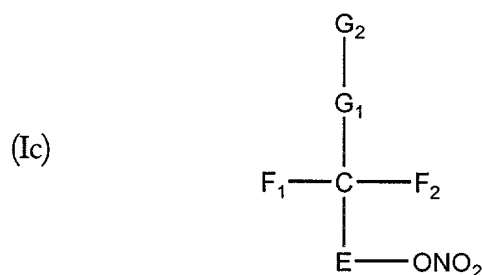
in which  $F_2$  is an organic radical which may be joined in a cyclic ring system with  $G_2$ , and which may contain inorganic counterions; E and  $G_1$  are both methylene groups;  $F_1$  is H; and  $G_2$  is  $R^N-Z^N$ ;

wherein  $R^N$  is an organic radical possessing a heteroaryl group containing P or S atoms where said P or S are positioned  $\beta$ ,  $\gamma$ , or  $\delta$  to a nitrate group as identified in formula I; and  $Z^N$  is  $W^N_{mm}-X^N_{nn}-Y^N_{oo}$ ;

wherein mm, nn and oo are 0 or 1; and  $W^N$ ,  $X^N$ ,  $Y^N$  are NH,  $NR^{NN}$ , CO, O or  $CH_2$ ;

wherein  $R^{NN}$  is a short chain alkyl group ( $C_1 - C_{12}$ ).

3. A method for treating pain, treating inflammation or providing analgesia in a subject, comprising administering to a subject in need thereof an effective amount of a therapeutic compound, wherein the therapeutic compound is of the formula (Ic):



in which E is  $(R^1R^2C)_m$  and  $G_2-G_1-CF_1F_2-$  is  $R^{19}-(R^3R^4C)_p-(R^{17}R^{18}C)_n-$ ;

wherein: m, n, p are integers from 0 to 10;

$R^{3,17}$  are each independently hydrogen, a nitrate group, or A; and

$R^{1,4}$  are each independently hydrogen, or A;

where A is selected from a substituted or unsubstituted aliphatic group (preferably a branched or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain, which optionally may contain O, S,  $NR^6$  and unsaturations in the chain, optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; an unsubstituted or substituted cyclic aliphatic moiety having from 3 to 7 carbon atoms in the aliphatic ring, which optionally may contain O, S,  $NR^6$  and unsaturations in the ring, optionally bearing

from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; an unsubstituted or substituted aliphatic moiety constituting a linkage of from 0 to 5 carbons, between  $R^1$  and  $R^3$  and/or between  $R^{17}$  and  $R^4$ , which optionally may contain O, S,  $NR^6$  and unsaturations in the linkage, and optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups); a substituted or unsubstituted aliphatic group (preferably a branched, cyclic or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain) containing carbonyl linkages (e.g.,  $C=O$ ,  $C=S$ ,  $C=NOH$ ), which optionally may contain O, S,  $NR^6$  and unsaturations in the chain, optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; a substituted or unsubstituted aryl group; a heterocyclic group; amino (including alkylamino, dialkylamino (including cyclic amino, diamino and triamino moieties), arylamino, diarylamino, and alkylarylamino); hydroxy; alkoxy; a substituted or unsubstituted aryloxy;

wherein X is F, Br, Cl,  $NO_2$ ,  $CH_2$ ,  $CF_2$ , O, NH, NMe, CN,  $NHOH$ ,  $N_2H_3$ ,  $N_2H_2R^{13}$ ,  $N_2HR^{13}R^{14}$ ,  $N_3$ , S, SCN,  $SCN_2H_2(R^{15})_2$ ,  $SCN_2H_3(R^{15})$ ,  $SC(O)N(R^{15})_2$ ,  $SC(O)NHR^{15}$ ,  $SO_3M$ , SH,  $SR^7$ ,  $SO_2M$ ,  $S(O)R^8$ ,  $S(O)_2R^9$ ,  $S(O)OR^8$ ,  $S(O)_2OR^9$ ,  $PO_2HM$ ,  $PO_3HM$ ,  $PO_3M_2$ ,  $P(O)(OR^{15})(OR^{16})$ ,  $P(O)(OR^{16})(OM)$ ,  $P(O)(R^{15})(OR^8)$ ,  $P(O)(OM)R^{15}$ ,  $CO_2M$ ,  $CO_2H$ ,  $CO_2R^{11}$ ,  $C(O)$ ,  $C(O)R^{12}$ ,  $C(O)(OR^{13})$ ,  $PO_2H$ ,  $PO_2M$ ,  $P(O)(OR^{14})$ ,  $P(O)(R^{13})$ , SO,  $SO_2$ ,  $C(O)(SR^{13})$ ,  $SR^5$ ,  $SSR^7$  or  $SSR^5$ ;

Y is F, Br, Cl,  $CH_3$ ,  $CF_2H$ ,  $CF_3$ , OH,  $NH_2$ ,  $NHR^6$ ,  $NR^6R^7$ , CN,  $NHOH$ ,  $N_2H_3$ ,  $N_2H_2R^{13}$ ,  $N_2HR^{13}R^{14}$ ,  $N_3$ , S, SCN,  $SCN_2H_2(R^{15})_2$ ,  $SCN_2H_3(R^{15})$ ,  $SC(O)N(R^{15})_2$ ,  $SC(O)NHR^{15}$ ,  $SO_3M$ , SH,  $SR^7$ ,  $SO_2M$ ,  $S(O)R^8$ ,  $S(O)_2R^9$ ,  $S(O)OR^8$ ,  $S(O)_2OR^9$ ,  $PO_2HM$ ,  $PO_3M_2$ ,  $P(O)(OR^{15})(OR^{16})$ ,  $P(O)(OR^{16})(OM)$ ,  $P(O)(R^{15})(OR^8)$ ,  $P(O)(OM)R^{15}$ ,  $CO_2M$ ,  $CO_2H$ ,  $CO_2R^{11}$ ,  $C(O)R^{12}$ ,  $C(O)(OR^{13})$ ,  $C(O)(SR^{13})$ ,  $SR^5$ ,  $SSR^7$  or  $SSR^5$ , or does not exist;

$R^2$ ,  $R^5$ ,  $R^{18}$ ,  $R^{19}$  are optionally hydrogen, A or X-Y;

$R^6$ ,  $R^7$ ,  $R^8$ ,  $R^9$ ,  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ ,  $R^{14}$ ,  $R^{15}$ ,  $R^{16}$  are the same or different alkyl or acyl groups containing 1-24 carbon atoms which may contain 1-4  $ONO_2$  substituents; or  $C_1 - C_6$  connections to  $R^1 - R^4$  in cyclic derivatives which may contain 1-4  $ONO_2$  substituents; or are each independently hydrogen a nitrate group or A;

M is H, Na<sup>+</sup>, K<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, N<sup>+</sup>H<sub>k</sub>R<sup>11</sup><sub>(4-k)</sub> where k is 0-3; or other pharmaceutically acceptable counterion;

and with the proviso that when m = n = p = 1 and R<sup>19</sup>, R<sup>2</sup>, R<sup>18</sup>, R<sup>1</sup> = H and R<sup>17</sup>, R<sup>3</sup> are nitrate groups, R<sup>4</sup> is not H.

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4. The method of claim 1, wherein F<sub>2</sub> is a nitrate group; and E, F<sub>1</sub>, G<sub>1</sub>, G<sub>2</sub> are the same or different organic radicals which may be joined in cyclic ring systems, and which may contain inorganic counterions;

with the proviso that when E and G<sub>1</sub> are methylene groups and F<sub>1</sub> is H, G<sub>2</sub> is not a nitrate group, nor R<sup>N</sup>-Z<sup>N</sup>;

10

wherein R<sup>N</sup> is any aryl or heteroaryl group and Z<sup>N</sup> is (CO)<sub>mm</sub>-X<sup>N</sup><sub>nn</sub>-Y<sup>N</sup><sub>oo</sub>;

wherein mm, nn, oo are 0 or 1 and X<sup>N</sup>, Y<sup>N</sup> are NH, NR<sup>NN</sup>, O or CH<sub>2</sub>;

wherein R<sup>NN</sup> is a short chain alkyl group (C<sub>1</sub> - C<sub>12</sub>).

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5. The method of claim 2, wherein F<sub>2</sub> is a nitrate group; E and G<sub>1</sub> are methylene groups; F<sub>1</sub> is H; and G<sub>2</sub> is R<sup>N</sup>-Z<sup>N</sup>;

wherein R<sup>N</sup> is an organic radical possessing an heteroaryl group containing P or S atoms where said P or S are positioned β, γ, or δ to a nitrate group as identified in formula I; and Z<sup>N</sup> is W<sup>N</sup><sub>mm</sub>-X<sup>N</sup><sub>nn</sub>-Y<sup>N</sup><sub>oo</sub>;

20

wherein mm, nn, oo are 0 or 1 and W<sup>N</sup>, X<sup>N</sup>, Y<sup>N</sup> are NH, NR<sup>NN</sup>, CO, O or CH<sub>2</sub>;

wherein R<sup>NN</sup> is a short chain alkyl group (C<sub>1</sub> - C<sub>12</sub>).

6. The method of claim 3, wherein R<sup>19</sup> is X-Y.

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7. The method of claim 6, wherein:

R<sup>1</sup> and R<sup>3</sup> are the same or different and selected from H and C<sub>1</sub>-C<sub>4</sub> alkyl chains, which chains may include one O linking R<sup>1</sup> and R<sup>3</sup> to form pentosyl, hexosyl, cyclopentyl, or cyclohexyl rings, which rings may optionally bear hydroxyl substituents;

$R^2$  and  $R^4$  are the same or different and selected from H, a nitrate group,  $C_1$ - $C_4$  alkyl chains optionally bearing 1-3 nitrate groups, and acyl groups ( $-C(O)R^5$ );

$R^7$ ,  $R^{11}$  are the same or different  $C_1 - C_8$  alkyl or acyl;

$R^5$ ,  $R^6$ ,  $R^8$ ,  $R^9$ ,  $R^{12}$ ,  $R^{13}$ ,  $R^{14}$ ,  $R^{15}$ ,  $R^{16}$  are the same or different and are alkyl groups containing 1-12 carbon atoms which may contain 1-4  $ONO_2$  substituents; or  $C_1$  or  $C_2$  connections to  $R^1 - R^3$  in cyclic derivatives; and

M is H,  $Na^+$ ,  $K^+$ ,  $NH_4^+$  or  $N^+H_kR^{11}_{(4-k)}$ , where k is 0-3.

8. The method of claim 7, wherein  $m = 1$ ,  $n = 0$ ,  $p = 1$ .

9. The method of claim 8, wherein:

X is  $CH_2$ , O, NH, NMe, CN, NHOH,  $N_2H_3$ ,  $N_2H_2R^{13}$ ,  $N_2HR^{13}R^{14}$ ,  $N_3$ , S, SCN,  $SCN_2H_2(R^{15})_2$ ,  $SCN_2H_3(R^{15})$ ,  $SC(O)N(R^{15})_2$ ,  $SC(O)NHR^{15}$ ,  $SO_3M$ , SH,  $SR^7$ ,  $SO_2M$ ,  $S(O)R^8$ ,  $S(O)_2R^9$ ,  $S(O)OR^8$ ,  $S(O)_2OR^9$ ,  $PO_3HM$ ,  $PO_3M_2$ ,  $P(O)(OR^{15})(OR^{16})$ ,  $P(O)(OR^{16})(OM)$ ,  $P(O)(R^{15})(OR^8)$ ,  $P(O)(OM)R^{15}$ ,  $CO_2M$ ,  $CO_2H$ ,  $CO_2R^{11}$ ,  $C(O)$ ,  $C(O)R^{12}$ ,  $C(O)(OR^{13})$ ,  $PO_2M$ ,  $P(O)(OR^{14})$ ,  $P(O)(R^{13})$ , SO,  $SO_2$ ,  $C(O)(SR^{13})$ , or  $SSR^4$ ; and

Y is CN,  $N_2H_2R^{13}$ ,  $N_2HR^{13}R^{14}$ ,  $N_3$ , SCN,  $SCN_2H_2(R^{15})_2$ ,  $SC(O)N(R^{15})_2$ ,  $SC(O)NHR^{15}$ ,  $SO_3M$ ,  $SR^4$ ,  $SO_2M$ ,  $PO_3HM$ ,  $PO_3M_2$ ,  $P(O)(OR^{15})(OR^{16})$ ,  $P(O)(OR^{16})(OM)$ ,  $P(O)(R^{15})(OR^8)$ ,  $P(O)(OM)R^{15}$ ,  $CO_2M$ ,  $CO_2H$ ,  $CO_2R^{11}$ ,  $C(O)R^{12}$ ,  $C(O)(SR^{13})$ ,  $SR^5$ , or  $SSR^5$ , or does not exist.

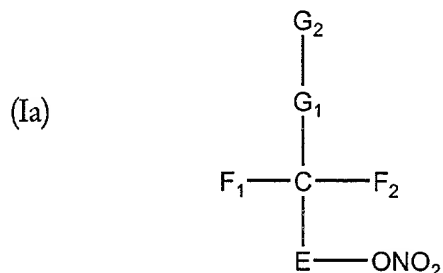
10. The method of claim 8, wherein:

$R^5$ ,  $R^6$ ,  $R^8$ ,  $R^9$ ,  $R^{12}$ ,  $R^{13}$ ,  $R^{14}$ ,  $R^{15}$ ,  $R^{16}$  are the same or different and are alkyls containing 1-12 carbon atoms; or  $C_1$  or  $C_2$  connections to  $R^1$  or  $R^3$  in cyclic derivatives;

X is  $CH_2$ , O, NH, NMe, S,  $SO_3M$ , SH,  $SR^7$ ,  $SO_2M$ ,  $S(O)R^8$ ,  $S(O)_2R^9$ ,  $S(O)OR^8$ ,  $S(O)_2OR^9$ ,  $PO_3M_2$ ,  $P(O)(OR^{15})(OR^{16})$ ,  $P(O)(OR^{16})(OM)$ ,  $P(O)(R^{15})(OR^8)$ ,  $PO_3HM$  or  $P(O)(OM)R^{15}$ ; and

Y is  $SO_2M$ ,  $SO_3M$ ,  $PO_3HM$ ,  $PO_3M_2$ ,  $P(O)(OR^{15})(OR^{16})$ ,  $P(O)(OR^{16})(OM)$ ,  $SR^5$ ,  $SR^4$  or  $SSR^5$ , or does not exist.

11. A method for providing sedation, mitigating anxiety or providing anaesthesia in a subject in need thereof, comprising administering to a subject an effective amount of a therapeutic compound, wherein the therapeutic compound is of the formula (Ia):



10 in which E, F<sub>1</sub>, F<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub> are the same or different organic radicals which may be joined in cyclic ring systems, and which may contain inorganic counterions, but which do not contain an organic nitrate group;

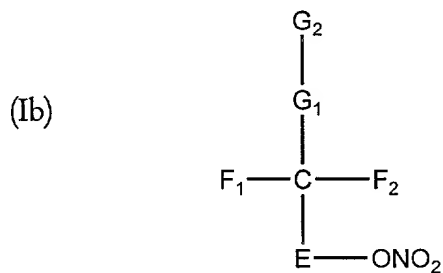
with the proviso that when E and G<sub>1</sub> are methylene groups and F<sub>1</sub> is H, G<sub>2</sub> is not a nitrate group, nor R<sup>N</sup>-Z<sup>N</sup>;

15 wherein R<sup>N</sup> is any aryl or heteroaryl group and Z<sup>N</sup> is (CO)<sub>mm</sub>-X<sup>N</sup><sub>nn</sub>-Y<sup>N</sup><sub>oo</sub>;

wherein mm, nn, oo are 0 or 1 and X<sup>N</sup>, Y<sup>N</sup> are NH, NR<sup>NN</sup>, O or CH<sub>2</sub>;

wherein R<sup>NN</sup> is a short chain alkyl group (C<sub>1</sub> - C<sub>12</sub>).

12. A method for providing sedation, mitigating anxiety or providing anaesthesia in a subject in need thereof, comprising administering to a subject an effective amount of a therapeutic compound, wherein the therapeutic compound is of the formula (Ib):



in which F<sub>2</sub> is an organic radical which may be joined in a cyclic ring system with G<sub>2</sub>,

and which may contain inorganic counterions, but is not a nitrate group; E and G<sub>1</sub> are methylene groups; F<sub>1</sub> is H; and G<sub>2</sub> is R<sup>N</sup>-Z<sup>N</sup>-;

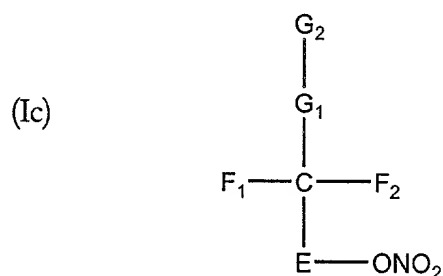
wherein R<sup>N</sup> is an organic radical possessing a heteroaryl group containing P or S atoms where said P or S are positioned β, γ, or δ to a nitrate group as identified in formula I; and Z<sup>N</sup>

5 is W<sup>N</sup><sub>mm</sub>-X<sup>N</sup><sub>nn</sub>-Y<sup>N</sup><sub>oo</sub>;

wherein mm, nn, oo are 0 or 1 and W<sup>N</sup>, X<sup>N</sup>, Y<sup>N</sup> are NH, NR<sup>NN</sup>, CO, O or CH<sub>2</sub>;

wherein R<sup>NN</sup> is a short chain alkyl group (C<sub>1</sub> - C<sub>12</sub>).

13. A method for providing sedation, mitigating anxiety or providing anaesthesia in a  
10 subject in need thereof, comprising administering to a subject an effective amount of a therapeutic compound, wherein the therapeutic compound is of the formula (Ic):



in which E is (R<sup>1</sup>R<sup>2</sup>C)<sub>m</sub> and G<sub>2</sub>-G<sub>1</sub>-CF<sub>1</sub>F<sub>2</sub>- is R<sup>19</sup>-(R<sup>3</sup>R<sup>4</sup>C)<sub>p</sub>-(R<sup>17</sup>R<sup>18</sup>C)<sub>n</sub>-;

wherein: m, n, p are integers from 0 to 10;

R<sup>3,17</sup> are each independently hydrogen, a nitrate group, or A; and

R<sup>1,4</sup> are each independently hydrogen, or A;

where A is selected from a substituted or unsubstituted aliphatic group (preferably a branched or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain, which optionally may contain O, S, NR<sup>6</sup> and unsaturations in the chain, optionally bearing  
25 from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; an unsubstituted or substituted cyclic aliphatic moiety having from 3 to 7 carbon atoms in the aliphatic ring, which optionally may contain O, S, NR<sup>6</sup> and unsaturations in the ring, optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; an unsubstituted or substituted aliphatic moiety constituting a linkage of from 0 to 5 carbons, between R<sup>1</sup> and R<sup>3</sup>

and/or between  $R^{17}$  and  $R^4$ , which optionally may contain O, S,  $NR^6$  and unsaturations in the linkage, and optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups); a substituted or unsubstituted aliphatic group (preferably a branched, cyclic or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain) containing  
 5 carbonyl linkages (e.g.,  $C=O$ ,  $C=S$ ,  $C=NOH$ ), which optionally may contain O, S,  $NR^6$  and unsaturations in the chain, optionally bearing from 1 to 4 hydroxy, nitrate, amino, aryl, or heterocyclic groups; a substituted or unsubstituted aryl group; a heterocyclic group; amino (including alkylamino, dialkylamino (including cyclic amino, diamino and triamino moieties), arylamino, diarylamino, and alkylarylamino); hydroxy; alkoxy; a substituted or unsubstituted  
 10 aryloxy;

wherein X is F, Br, Cl,  $NO_2$ ,  $CH_2$ ,  $CF_2$ , O, NH, NMe, CN, NHOH,  $N_2H_3$ ,  $N_2H_2R^{13}$ ,  $N_2HR^{13}R^{14}$ ,  $N_3$ , S, SCN,  $SCN_2H_2(R^{15})_2$ ,  $SCN_2H_3(R^{15})$ ,  $SC(O)N(R^{15})_2$ ,  $SC(O)NHR^{15}$ ,  $SO_3M$ , SH,  $SR^7$ ,  $SO_2M$ ,  $S(O)R^8$ ,  $S(O)_2R^9$ ,  $S(O)OR^8$ ,  $S(O)_2OR^9$ ,  $PO_2HM$ ,  $PO_3HM$ ,  $PO_3M_2$ ,  $P(O)(OR^{15})(OR^{16})$ ,  $P(O)(OR^{16})(OM)$ ,  $P(O)(R^{15})(OR^8)$ ,  $P(O)(OM)R^{15}$ ,  $CO_2M$ ,  $CO_2H$ ,  $CO_2R^{11}$ ,  
 15  $C(O)$ ,  $C(O)R^{12}$ ,  $C(O)(OR^{13})$ ,  $PO_2H$ ,  $PO_2M$ ,  $P(O)(OR^{14})$ ,  $P(O)(R^{13})$ , SO,  $SO_2$ ,  $C(O)(SR^{13})$ ,  $SR^5$ ,  $SSR^7$  or  $SSR^5$ ;

Y is F, Br, Cl,  $CH_3$ ,  $CF_2H$ ,  $CF_3$ , OH,  $NH_2$ ,  $NHR^6$ ,  $NR^6R^7$ , CN, NHOH,  $N_2H_3$ ,  $N_2H_2R^{13}$ ,  $N_2HR^{13}R^{14}$ ,  $N_3$ , S, SCN,  $SCN_2H_2(R^{15})_2$ ,  $SCN_2H_3(R^{15})$ ,  $SC(O)N(R^{15})_2$ ,  $SC(O)NHR^{15}$ ,  $SO_3M$ , SH,  $SR^7$ ,  $SO_2M$ ,  $S(O)R^8$ ,  $S(O)_2R^9$ ,  $S(O)OR^8$ ,  $S(O)_2OR^9$ ,  $PO_2HM$ ,  $PO_3M_2$ ,  
 20  $P(O)(OR^{15})(OR^{16})$ ,  $P(O)(OR^{16})(OM)$ ,  $P(O)(R^{15})(OR^8)$ ,  $P(O)(OM)R^{15}$ ,  $CO_2M$ ,  $CO_2H$ ,  $CO_2R^{11}$ ,  $C(O)R^{12}$ ,  $C(O)(OR^{13})$ ,  $C(O)(SR^{13})$ ,  $SR^5$ ,  $SSR^7$  or  $SSR^5$ , or does not exist;

$R^2$ ,  $R^5$ ,  $R^{18}$ ,  $R^{19}$  are optionally hydrogen, A or X-Y;

$R^6$ ,  $R^7$ ,  $R^8$ ,  $R^9$ ,  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ ,  $R^{14}$ ,  $R^{15}$ ,  $R^{16}$  are the same or different alkyl or acyl groups containing 1-24 carbon atoms which may contain 1-4  $ONO_2$  substituents; or  $C_1 - C_6$

25 connections to  $R^1 - R^4$  in cyclic derivatives which may contain 1-4  $ONO_2$  substituents; or are each independently hydrogen a nitrate group or A;

M is H,  $Na^+$ ,  $K^+$ ,  $NH_4^+$ ,  $N^+H_kR^{11}_{(4-k)}$  where k is 0-3; or other pharmaceutically acceptable counterion;



and with the proviso that when  $m = n = p = 1$  and  $R^{19}, R^2, R^{18}, R^1 = H$  and  $R^{17}, R^3$  are nitrate groups,  $R^4$  is not H.

14. The method of claim 11, wherein  $F_2$  is a nitrate group; and  $E, F_1, G_1, G_2$  are the same or different organic radicals which may be joined in cyclic ring systems, and which may contain inorganic counterions;

with the proviso that when  $E$  and  $G_1$  are methylene groups and  $F_1$  is H,  $G_2$  is not a nitrate group, nor  $R^N-Z^N$ ;

wherein  $R^N$  is any aryl or heteroaryl group and  $Z^N$  is  $(CO)_{mm}-X_{nn}^N-Y_{oo}^N$ ;

wherein  $mm, nn, oo$  are 0 or 1 and  $X^N, Y^N$  are  $NH, NR^{NN}, O$  or  $CH_2$ ;

wherein  $R^{NN}$  is a short chain alkyl group ( $C_1 - C_{12}$ ).

15. The method of claim 12, wherein  $F_2$  is a nitrate group;  $E$  and  $G_1$  are methylene groups;  $F_1$  is H; and  $G_2$  is  $R^N-Z^N$ ;

wherein  $R^N$  is an organic radical possessing an heteroaryl group containing P or S atoms where said P or S are positioned  $\beta, \gamma$ , or  $\delta$  to a nitrate group as identified in formula I; and  $Z^N$  is  $W_{mm}^N-X_{nn}^N-Y_{oo}^N$ ;

wherein  $mm, nn, oo$  are 0 or 1 and  $W^N, X^N, Y^N$  are  $NH, NR^{NN}, CO, O$  or  $CH_2$ ;

wherein  $R^{NN}$  is a short chain alkyl group ( $C_1 - C_{12}$ ).

16. The method of claim 13, wherein  $R^{19}$  is X-Y.

17. The method of claim 16, wherein:

$R^1$  and  $R^3$  are the same or different and selected from H and  $C_1-C_4$  alkyl chains, which chains may include one O linking  $R^1$  and  $R^3$  to form pentosyl, hexosyl, cyclopentyl, or cyclohexyl rings, which rings may optionally bear hydroxyl substituents;

$R^2$  and  $R^4$  are the same or different and selected from H, a nitrate group,  $C_1-C_4$  alkyl chains optionally bearing 1-3 nitrate groups, and acyl groups ( $-C(O)R^5$ );

$R^7, R^{11}$  are the same or different  $C_1 - C_8$  alkyl or acyl;

$R^5, R^6, R^8, R^9, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}$  are the same or different and are alkyl groups containing 1-12 carbon atoms which may contain 1-4  $ONO_2$  substituents; or  $C_1$  or  $C_2$  connections to  $R^1 - R^3$  in cyclic derivatives; and

M is H,  $Na^+$ ,  $K^+$ ,  $NH_4^+$  or  $N^+H_kR^{11}_{(4-k)}$ , where k is 0-3.

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18. The method of claim 17, wherein  $m = 1, n = 0, p = 1$ .

19. The method of claim 18, wherein:

X is  $CH_2, O, NH, NMe, CN, NHOH, N_2H_3, N_2H_2R^{13}, N_2HR^{13}R^{14}, N_3, S, SCN,$   
 10  $SCN_2H_2(R^{15})_2, SCN_2H_3(R^{15}), SC(O)N(R^{15})_2, SC(O)NHR^{15}, SO_3M, SH, SR^7, SO_2M, S(O)R^8,$   
 $S(O)_2R^9, S(O)OR^8, S(O)_2OR^9, PO_3HM, PO_3M_2, P(O)(OR^{15})(OR^{16}), P(O)(OR^{16})(OM),$   
 $P(O)(R^{15})(OR^8), P(O)(OM)R^{15}, CO_2M, CO_2H, CO_2R^{11}, C(O), C(O)R^{12}, C(O)(OR^{13}), PO_2M,$   
 $P(O)(OR^{14}), P(O)(R^{13}), SO, SO_2, C(O)(SR^{13}),$  or  $SSR^4$ ; and

Y is  $CN, N_2H_2R^{13}, N_2HR^{13}R^{14}, N_3, SCN, SCN_2H_2(R^{15})_2, SC(O)N(R^{15})_2, SC(O)NHR^{15},$   
 15  $SO_3M, SR^4, SO_2M, PO_3HM, PO_3M_2, P(O)(OR^{15})(OR^{16}), P(O)(OR^{16})(OM), P(O)(R^{15})(OR^8),$   
 $P(O)(OM)R^{15}, CO_2M, CO_2H, CO_2R^{11}, C(O)R^{12}, C(O)(SR^{13}), SR^5,$  or  $SSR^5$ , or does not exist.

20. The method of claim 18, wherein:

$R^5, R^6, R^8, R^9, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}$  are the same or different and are alkyls containing 1-  
 20 12 carbon atoms; or  $C_1$  or  $C_2$  connections to  $R^1$  or  $R^3$  in cyclic derivatives;

X is  $CH_2, O, NH, NMe, S, SO_3M, SH, SR^7, SO_2M, S(O)R^8, S(O)_2R^9, S(O)OR^8,$   
 $S(O)_2OR^9, PO_3M_2, P(O)(OR^{15})(OR^{16}), P(O)(OR^{16})(OM), P(O)(R^{15})(OR^8), PO_3HM$  or  
 $P(O)(OM)R^{15}$ ; and

Y is  $SO_2M, SO_3M, PO_3HM, PO_3M_2, P(O)(OR^{15})(OR^{16}), P(O)(OR^{16})(OM), SR^5, SR^4$  or  
 25  $SSR^5$ , or does not exist.

21. The method of claim 3, with the proviso that when  $m = n = p = 1$  and  $R^{19}, R^2, R^{18}, R^1$   
 = H and  $R^{17}, R^3$  are nitrate groups,  $R^4$  is not  $C_1 - C_3$  alkyl.

22. The method of claim 13, with the proviso that when  $m = n = p = 1$  and  $R^{19}, R^2, R^{18}, R^1 = H$  and  $R^{17}, R^3$  are nitrate groups,  $R^4$  is not  $C_1 - C_3$  alkyl.

23. The method of any one of claims 1, 2, 3, 4 or 5, further comprising administering the therapeutic compound with a pharmaceutically acceptable vehicle.

24. The method of any one of claims 11, 12, 13, 14 or 15, further comprising administering the therapeutic compound with a pharmaceutically acceptable vehicle.

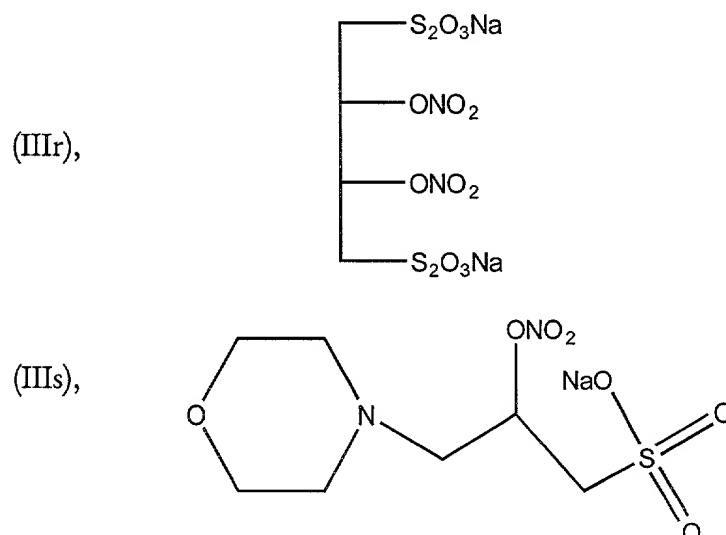
25. The method of any one of claims 1, 2, 3, 4 or 5, wherein the therapeutic compound modulates levels of the cyclic nucleotides cGMP and/or cAMP in said subject.

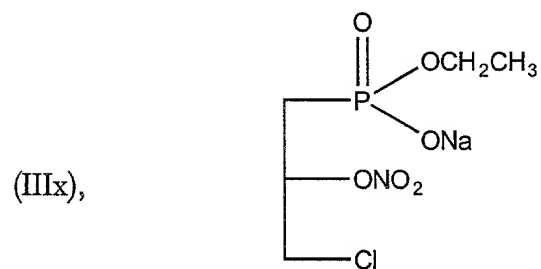
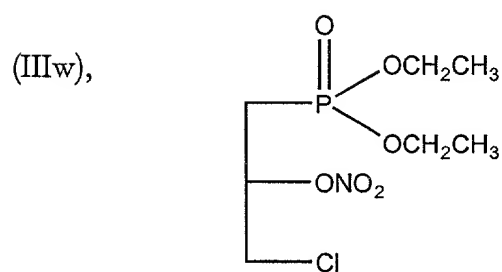
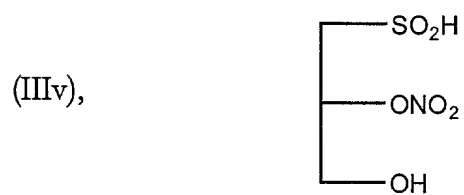
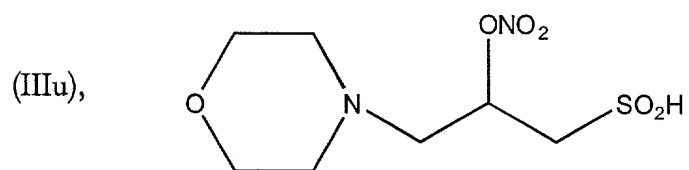
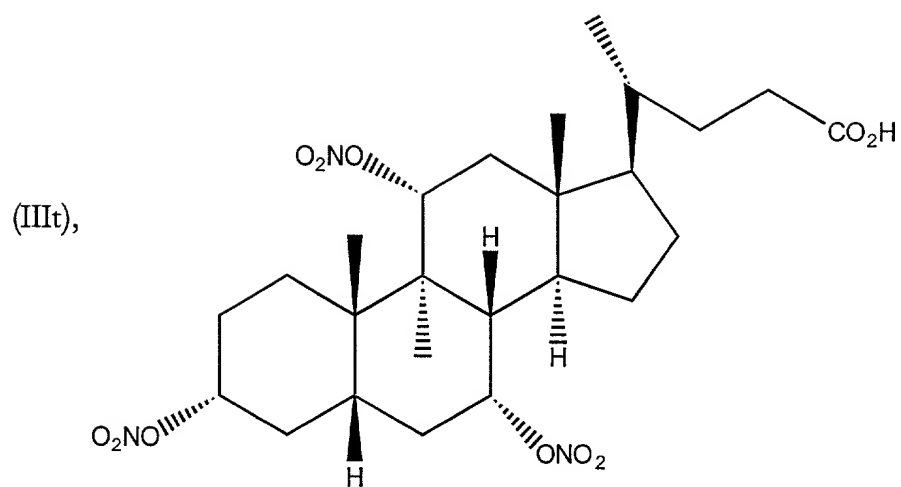
26. The method of any one of claims 11, 12, 13, 14 or 15, wherein the therapeutic compound modulates levels of the cyclic nucleotides cGMP and/or cAMP in said subject.

27. The method of any one of claims 1, 2, 3, 4 or 5, wherein the therapeutic compound modulates guanylyl cyclase activity in said subject.

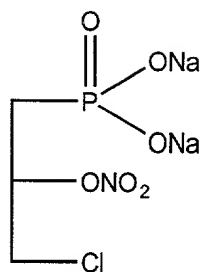
28. The method of any one of claims 11, 12, 13, 14 or 15, wherein the therapeutic compound modulates guanylyl cyclase activity in said subject.

29. A compound selected from the group consisting of

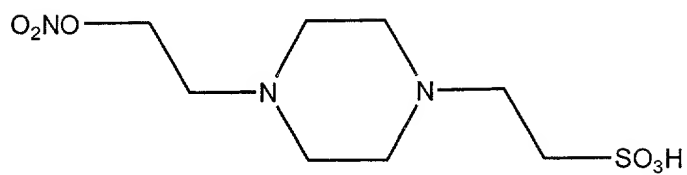




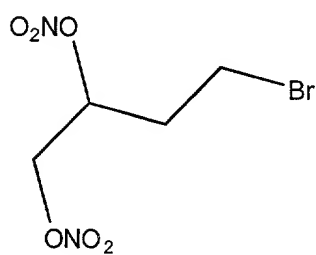
(IIIy),



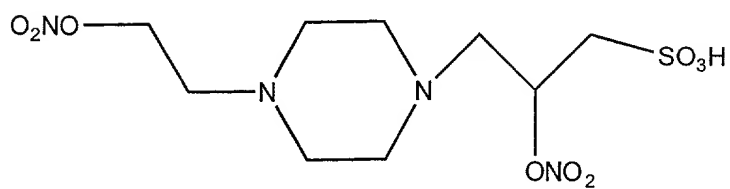
(IIIz),



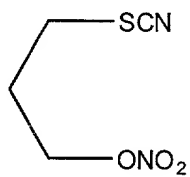
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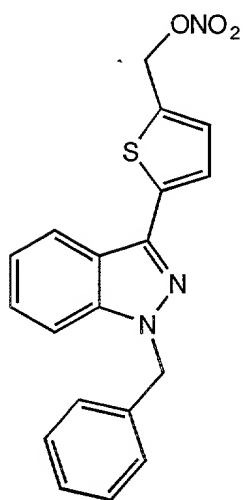
(IIIab),

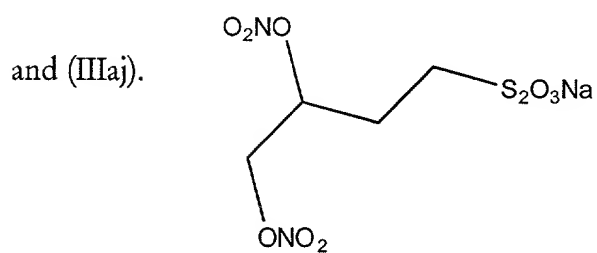
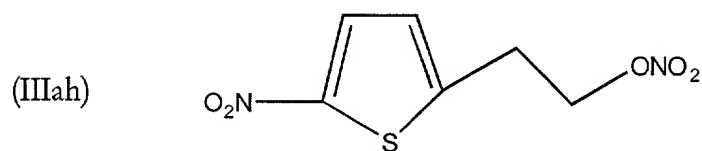
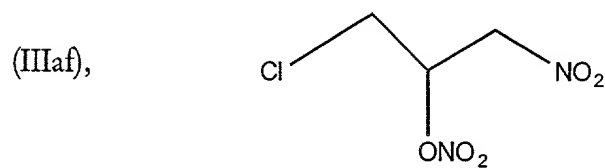
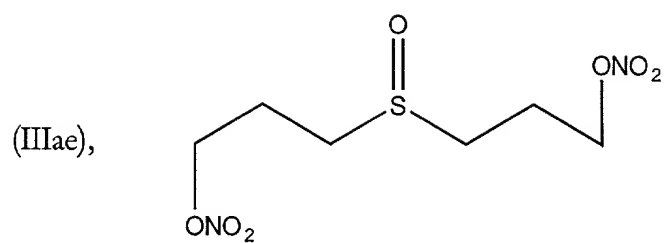


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(IIIad),

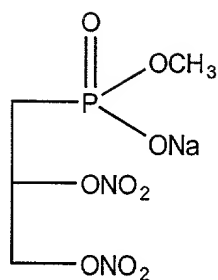




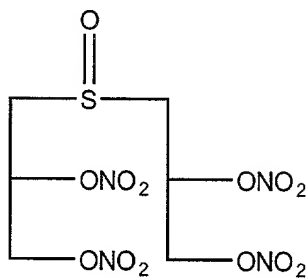
30. A compound selected from the group consisting of



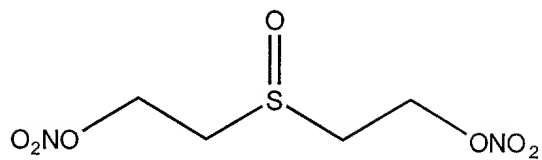
(IVo),



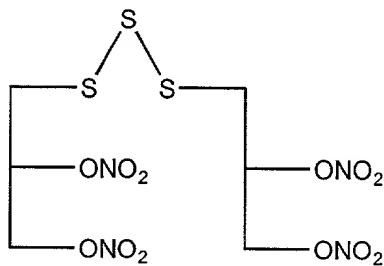
(IVp),



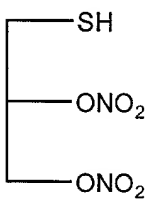
(IVq),



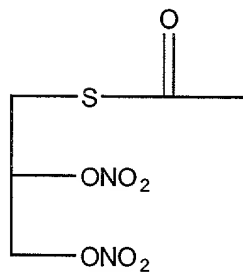
(IVr),



(IVs)

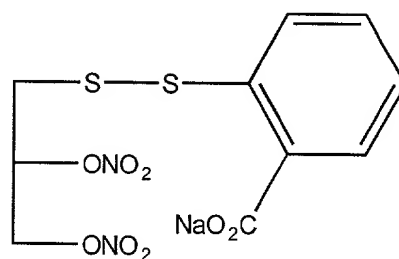


and (IVt).

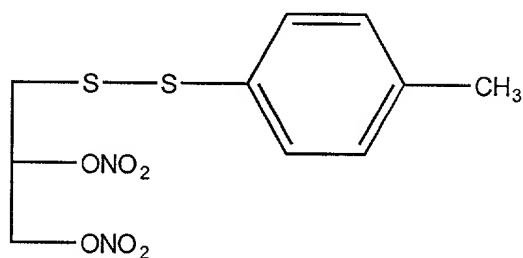


31. A compound selected from the group consisting of

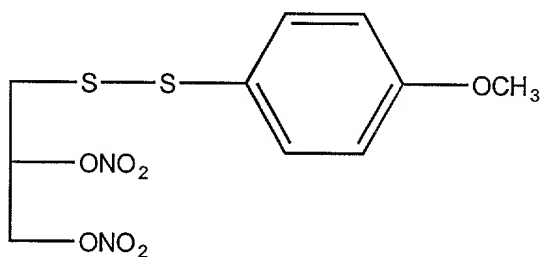
(Vd),



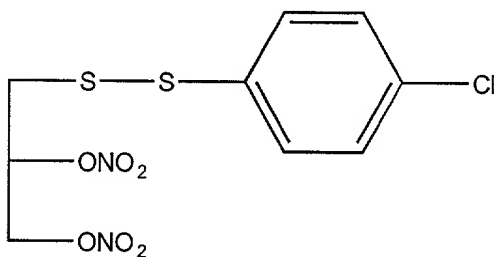
(Ve),



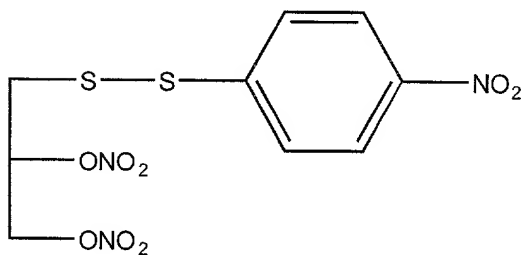
(Vf),



(Vg),

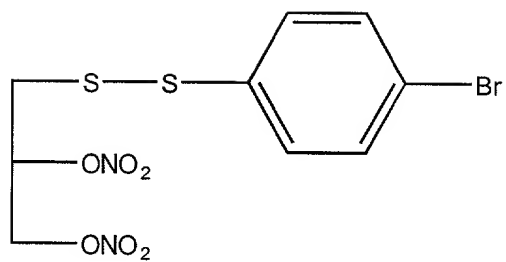


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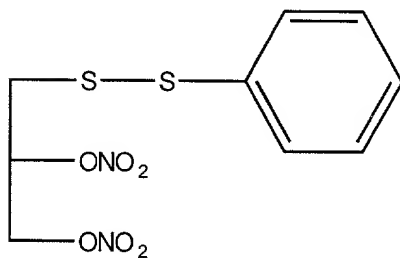




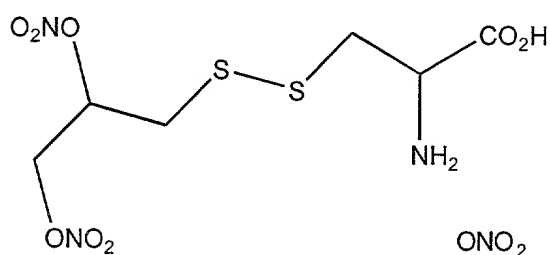
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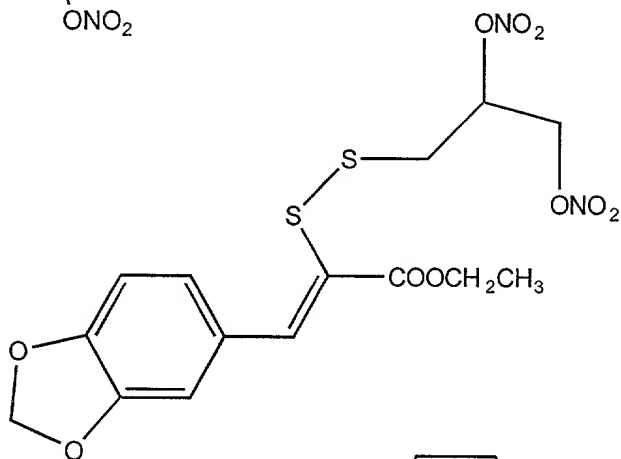
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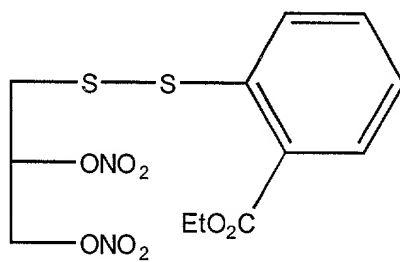
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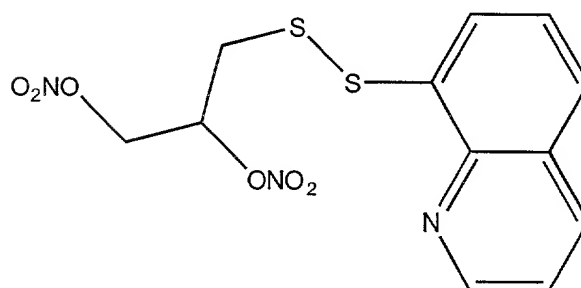
(Vl),

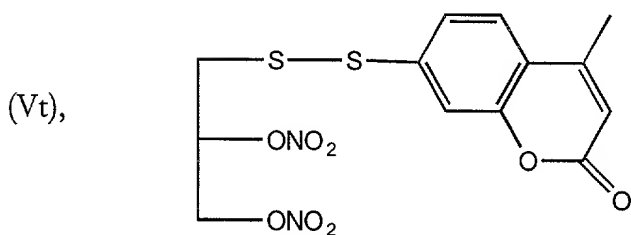
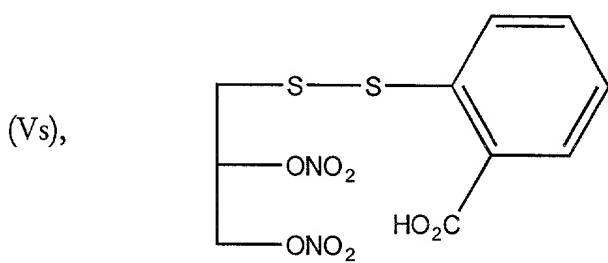
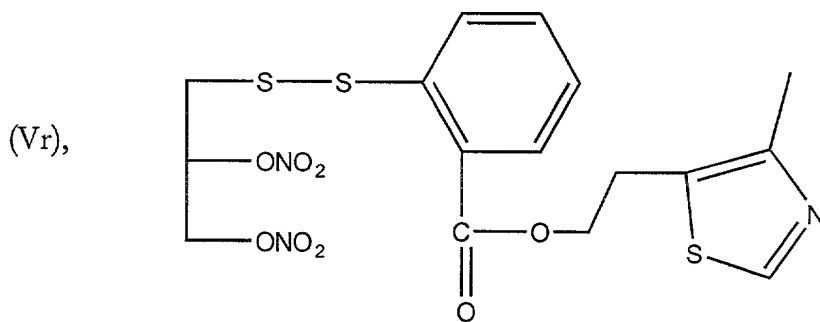
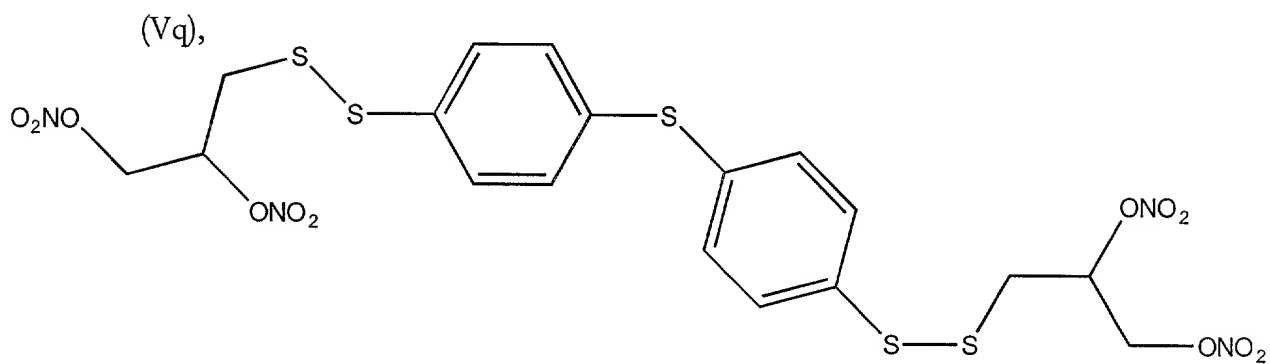
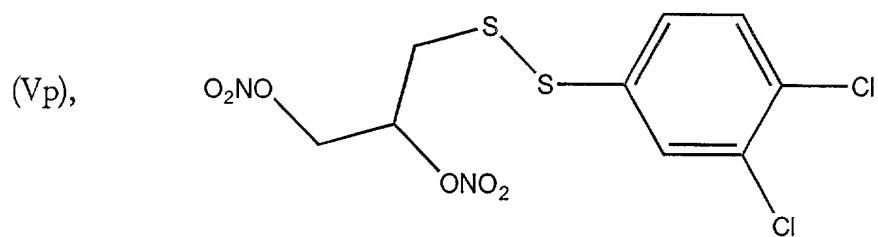
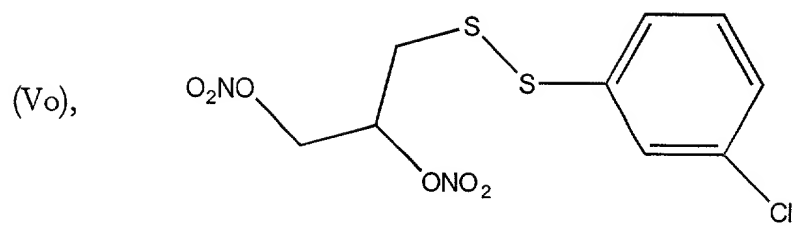


(Vm),

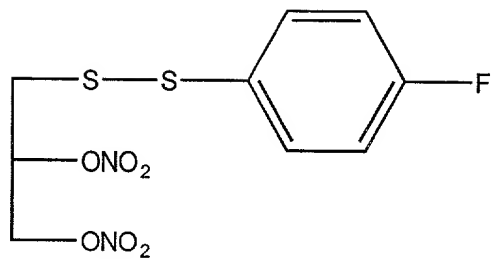


(Vn),



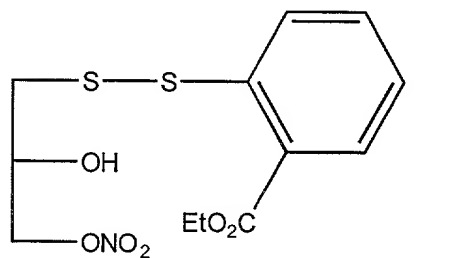


(Vu),



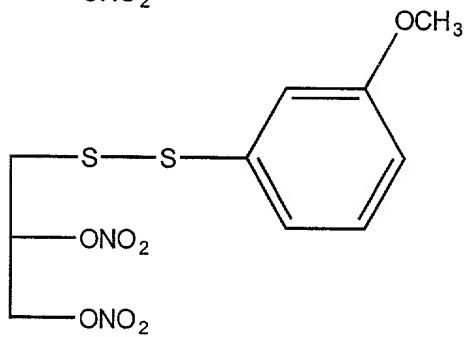
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(Vv),



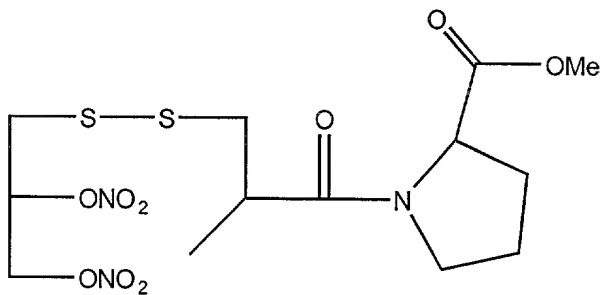
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(Vw),



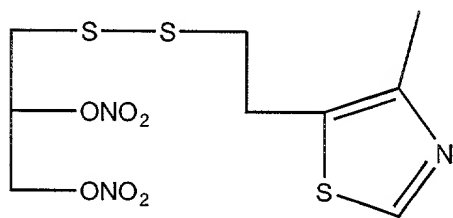
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(Vx),



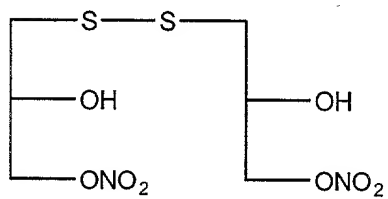
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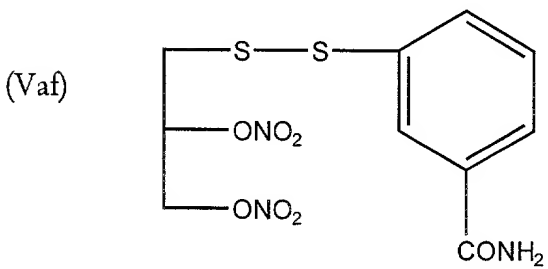
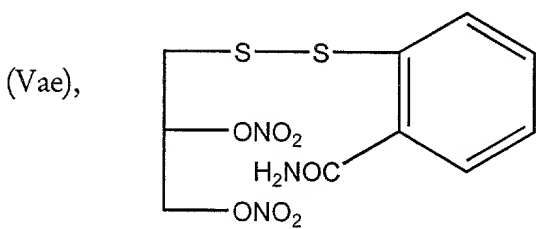
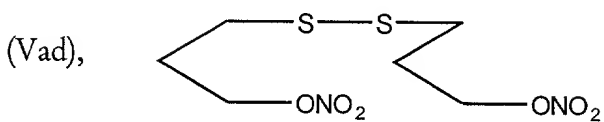
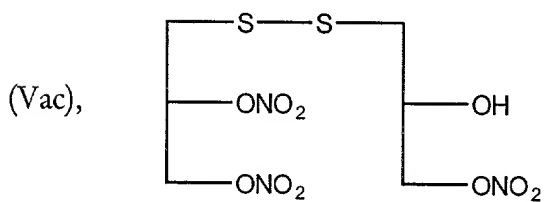
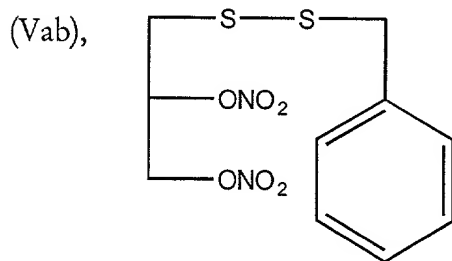
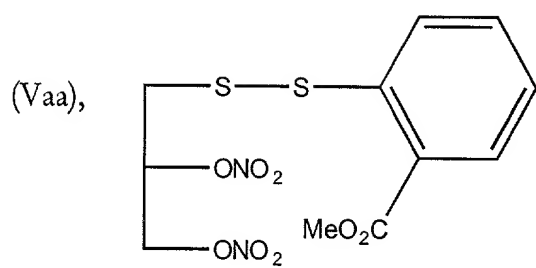
(Vy),



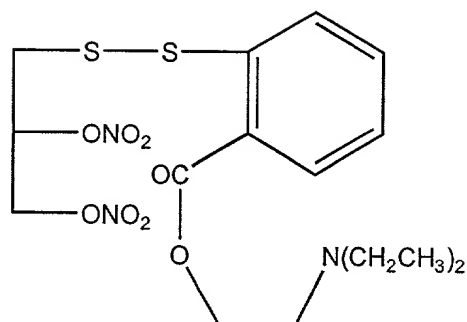
25

(Vz),





and (Vag).



5

32. A pharmaceutical composition comprising a said compound of any one of claims 29, 30 and 31 and a pharmaceutically acceptable vehicle.

10

## Abstract

Methods and therapeutic compounds for treating pain, mitigating inflammation, effecting analgesia and/or effecting sedation in a subject are described. A subject is administered an effective amount of a therapeutic compound which is a nitrate ester. Novel

5 pharmaceutical compositions are also described.

FIGURE 1

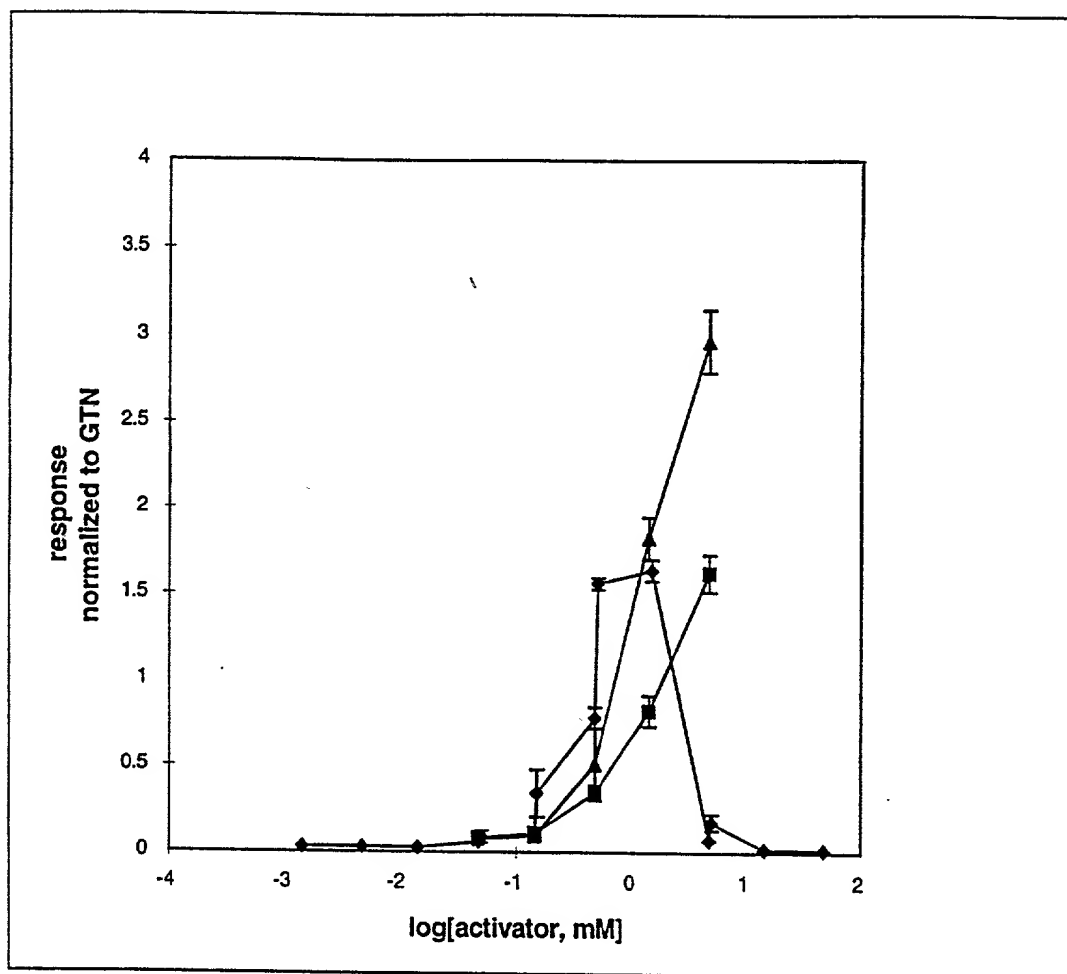


FIGURE 2

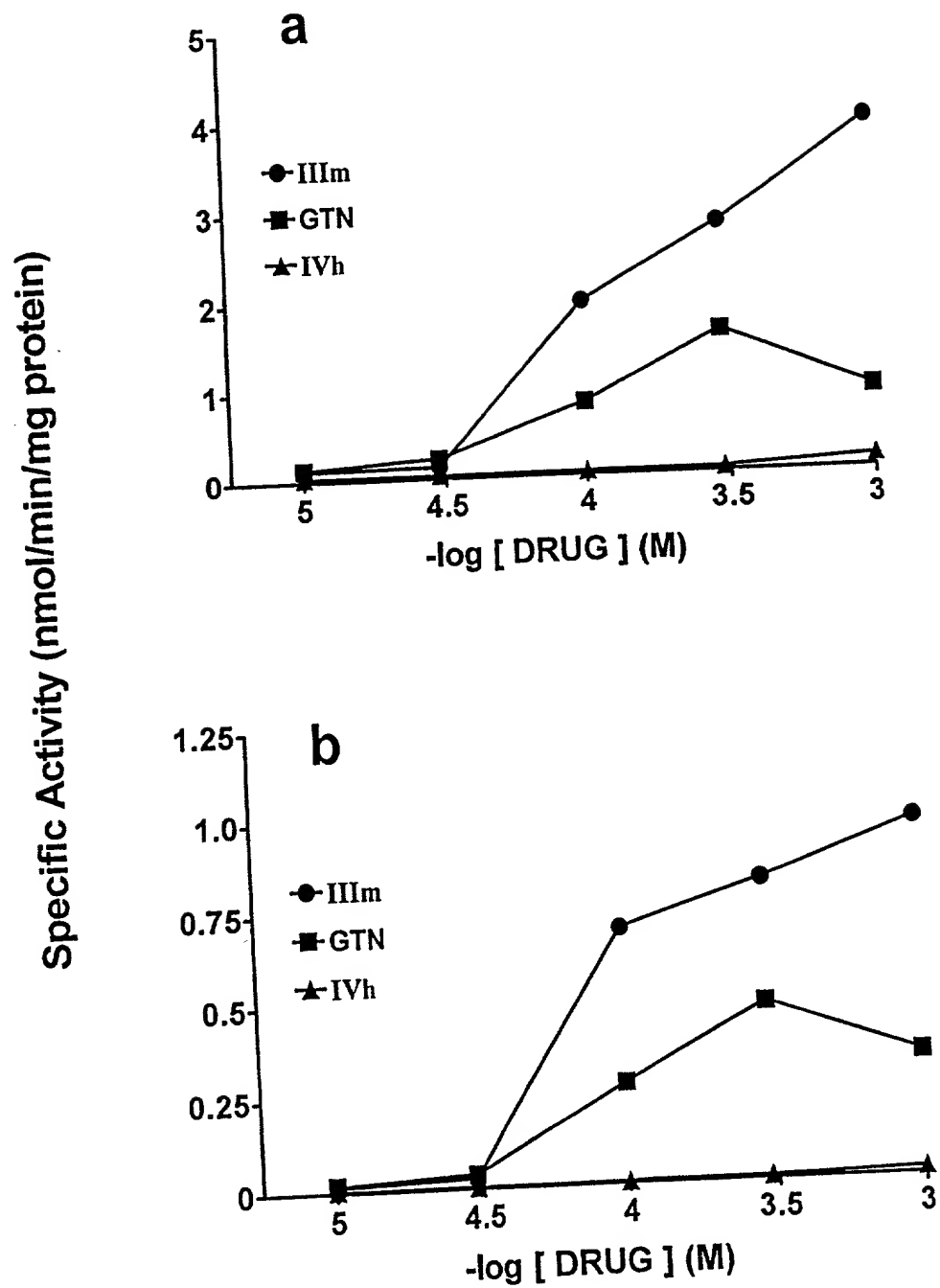




FIGURE 3

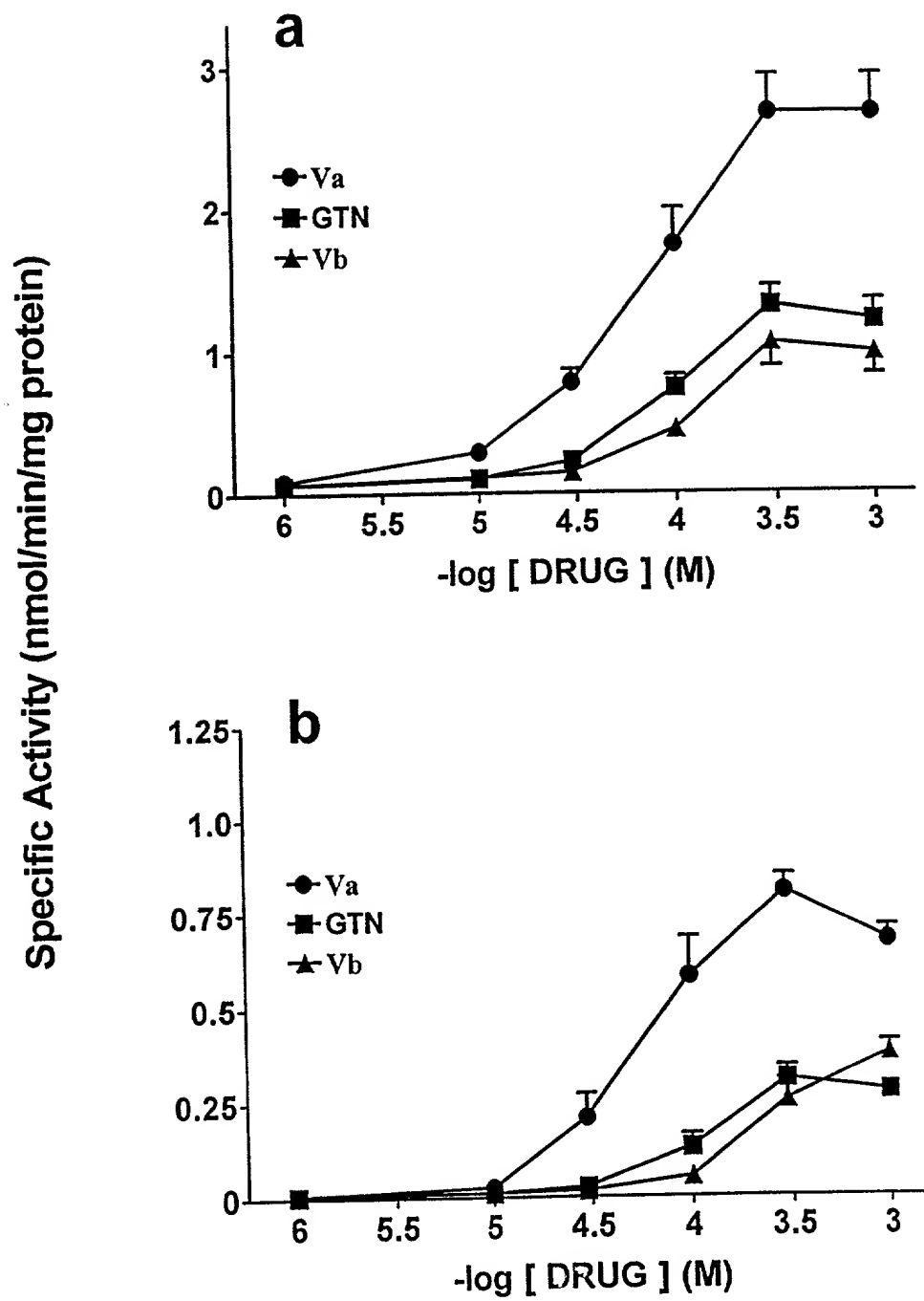


FIGURE 4

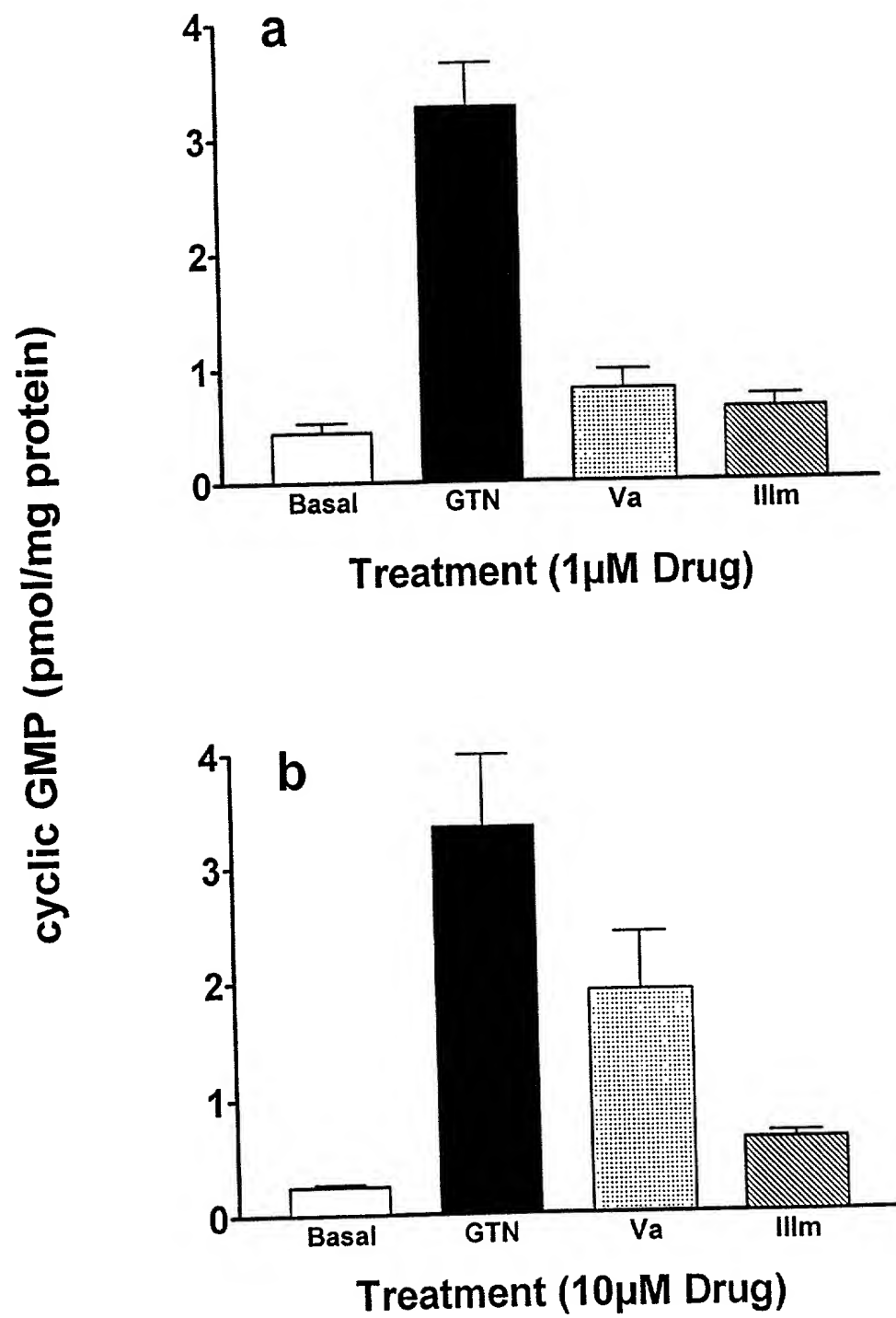


FIGURE 5

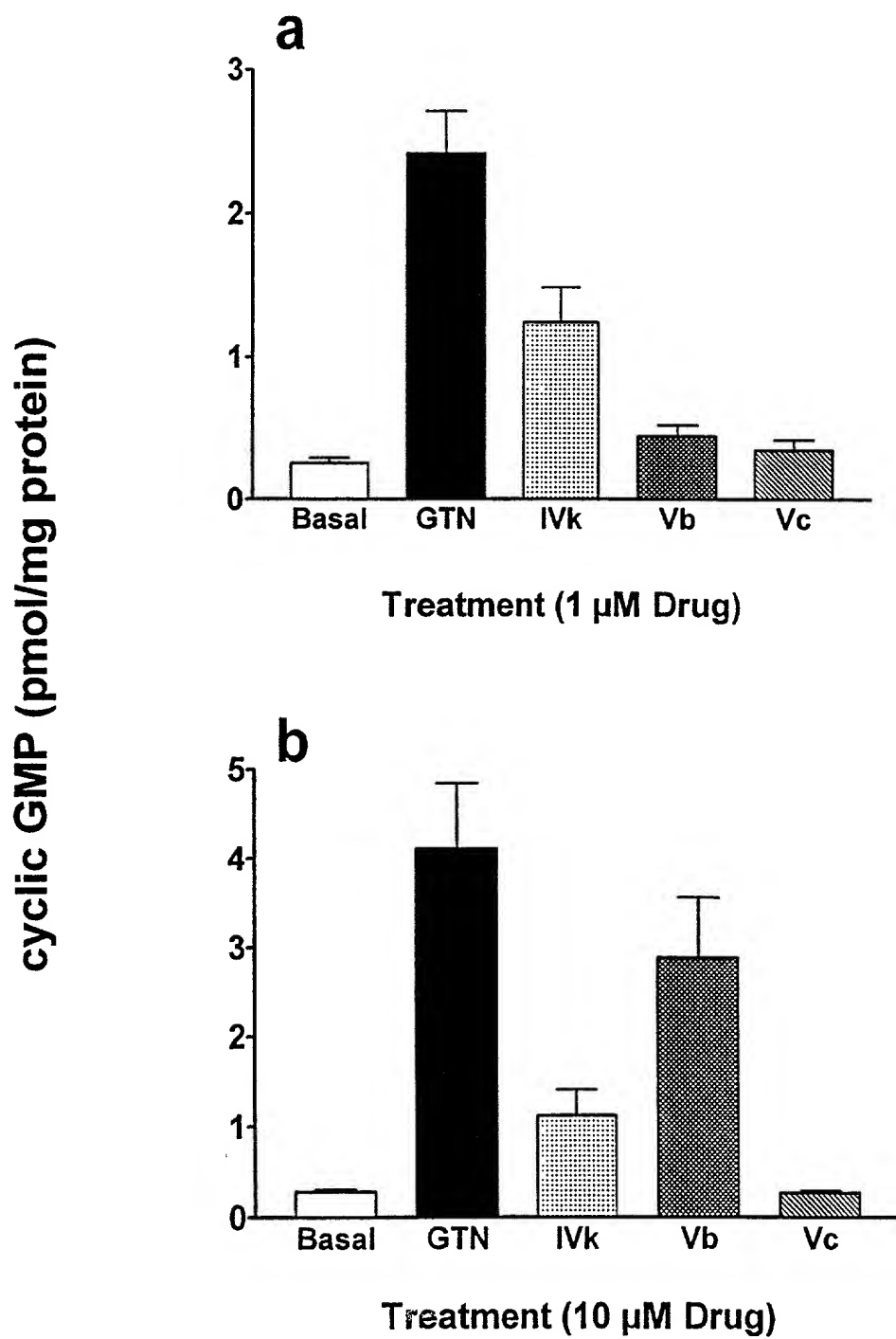


FIGURE 6

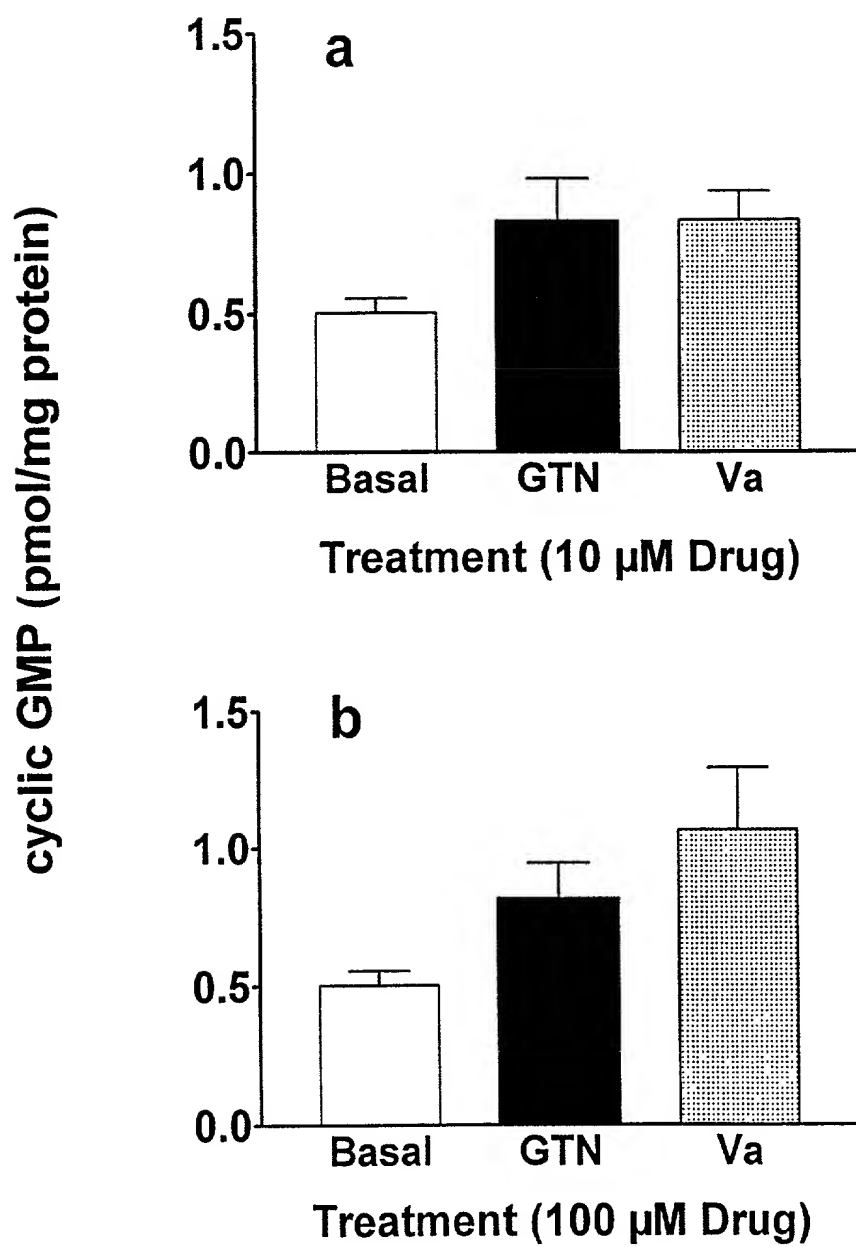


FIGURE 7

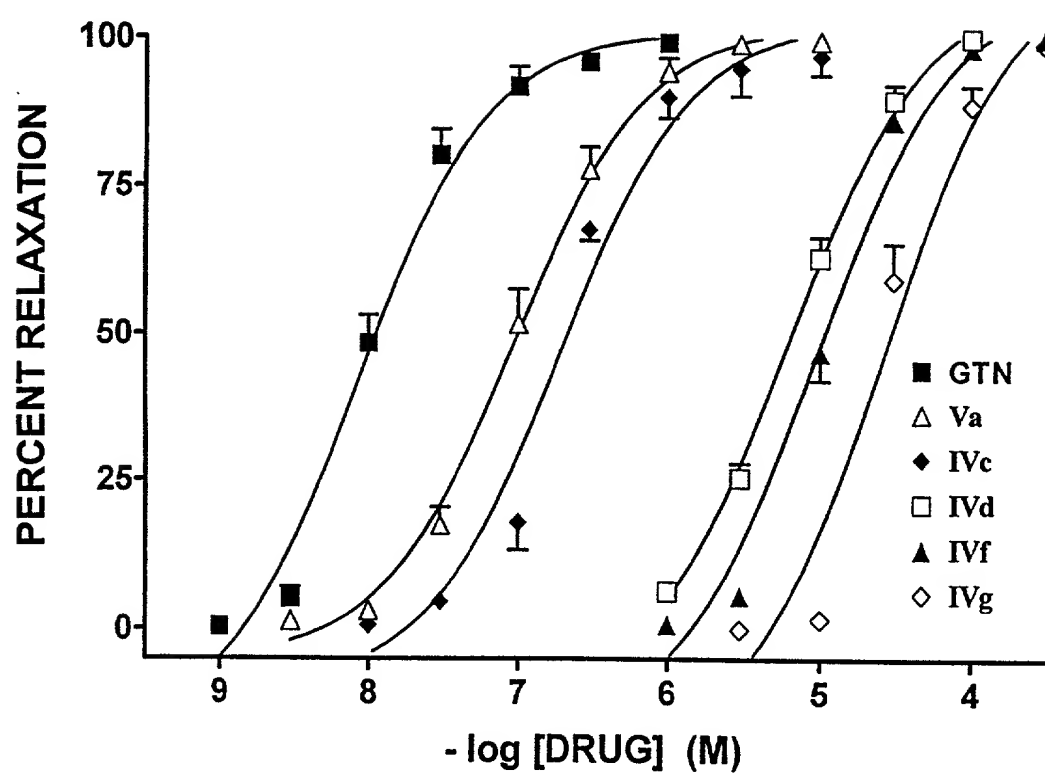


FIGURE 8

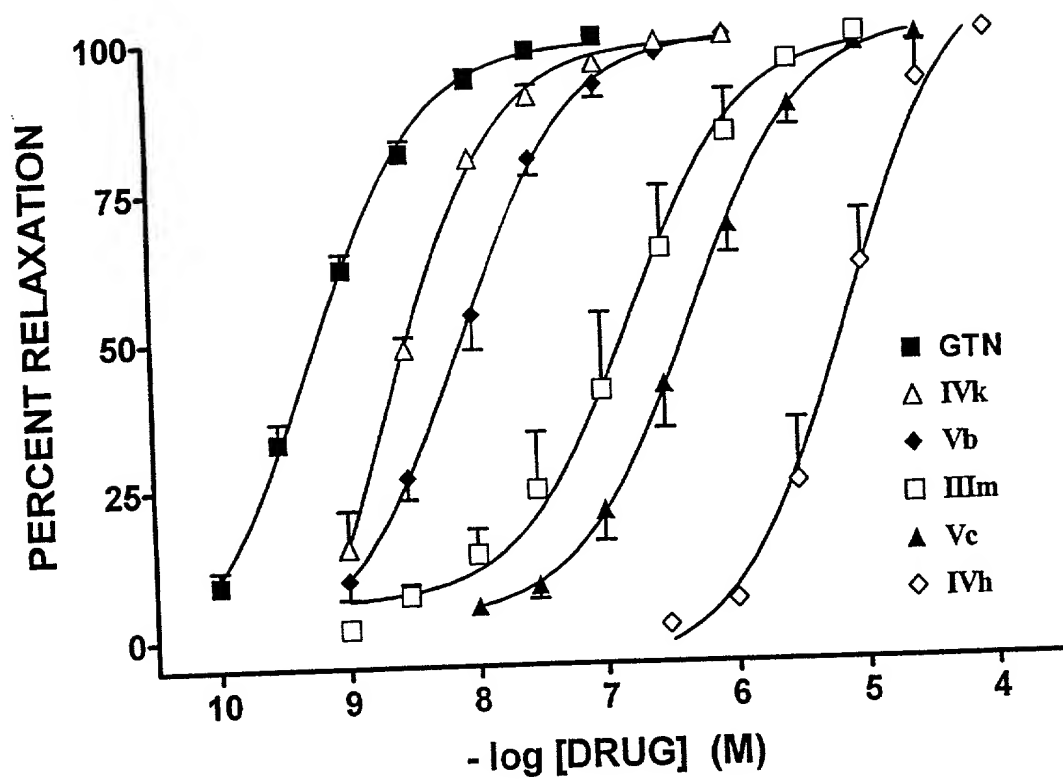


FIGURE 9

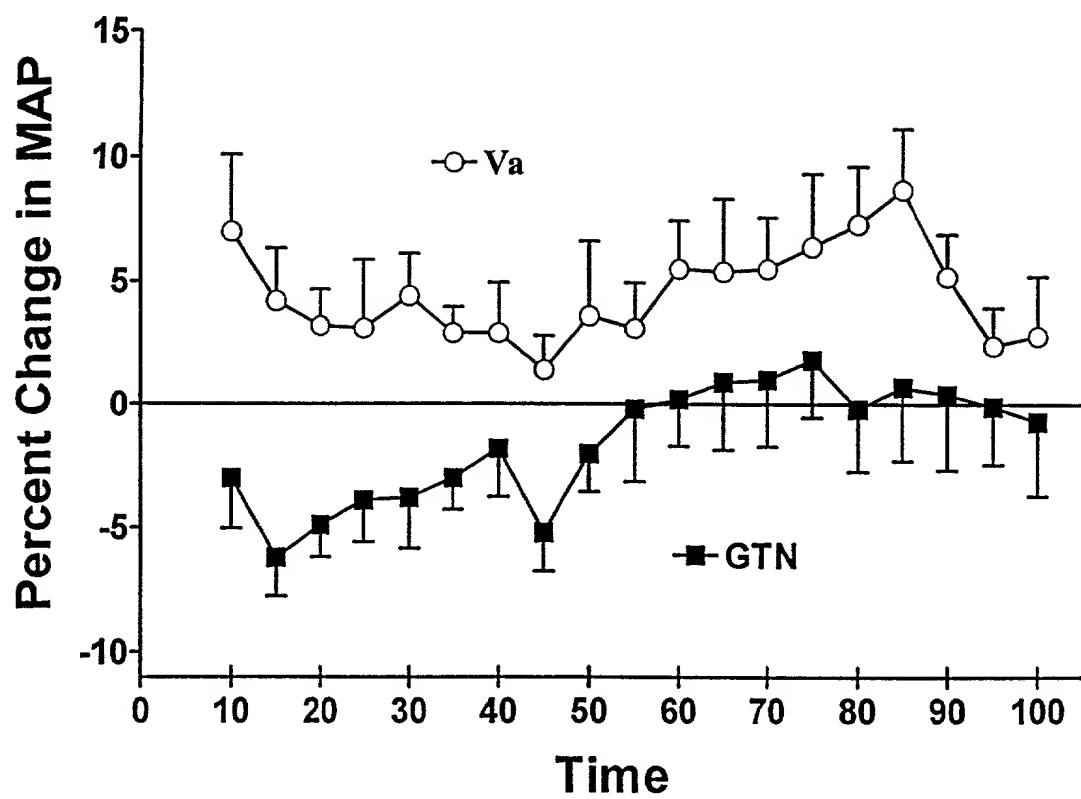


FIGURE 10

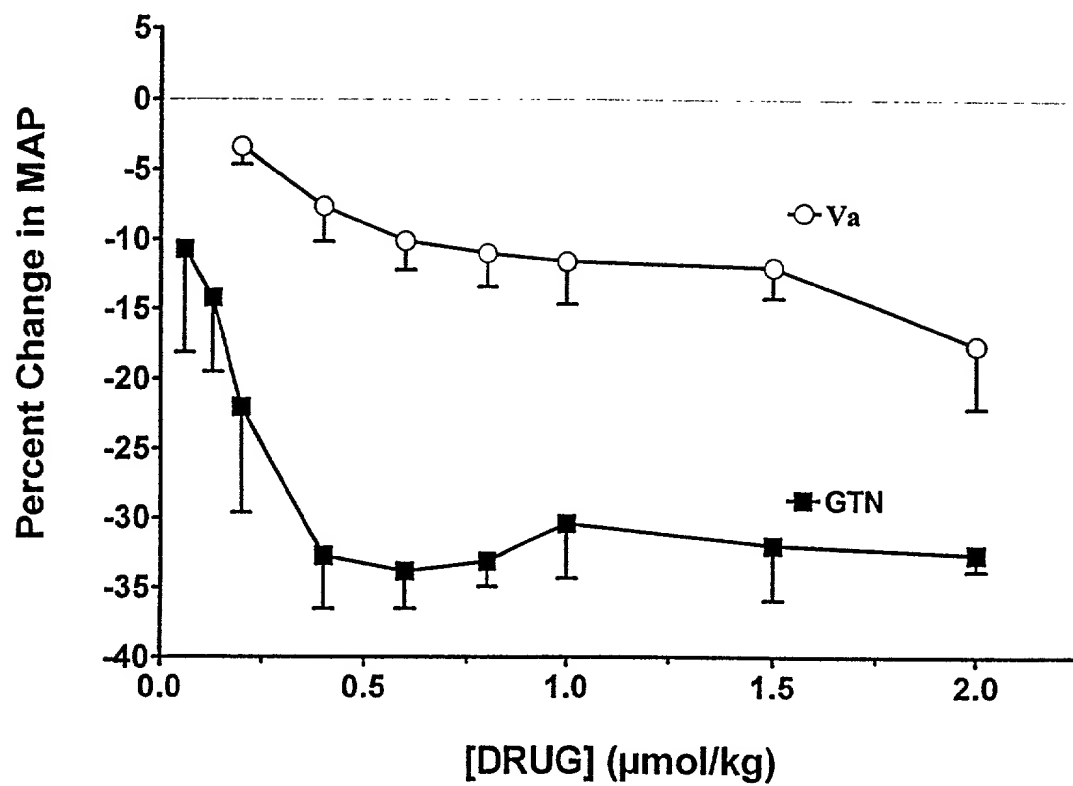




FIGURE 11

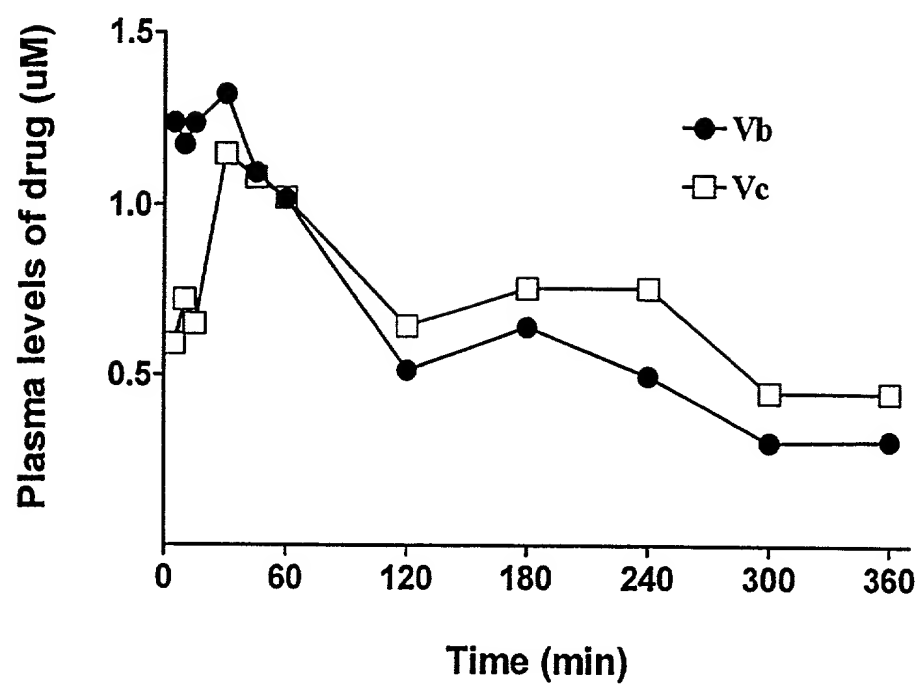


FIGURE 12

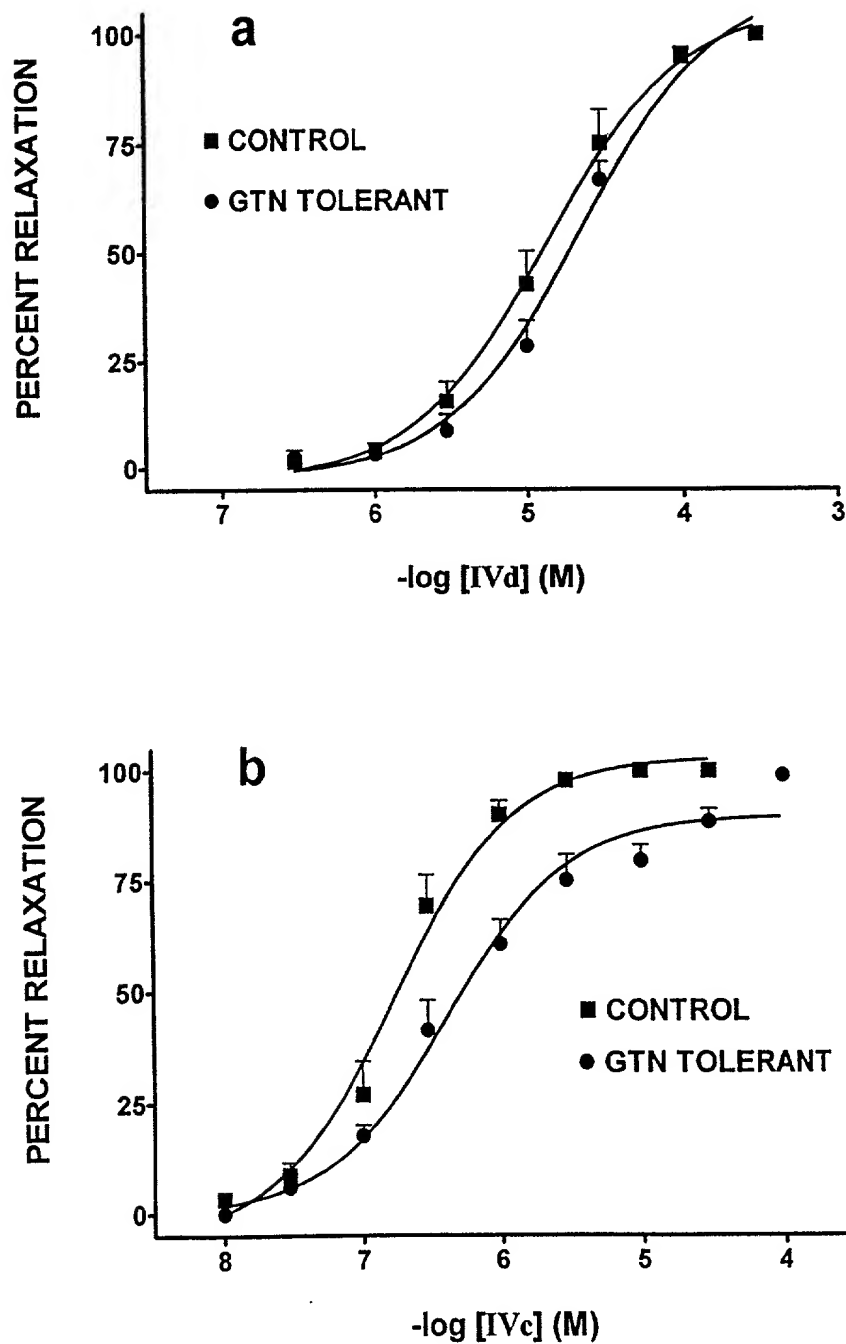


Figure 13

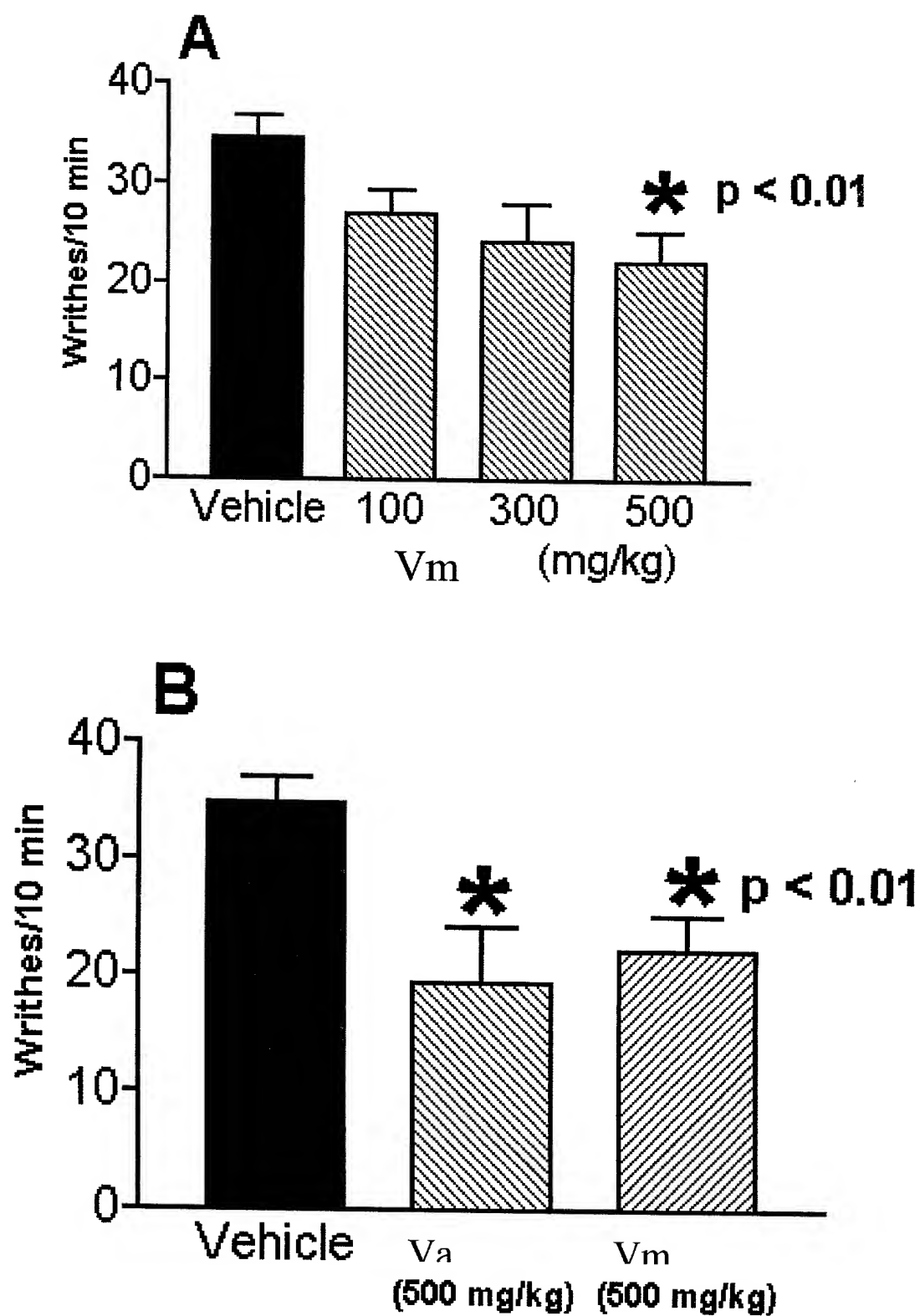
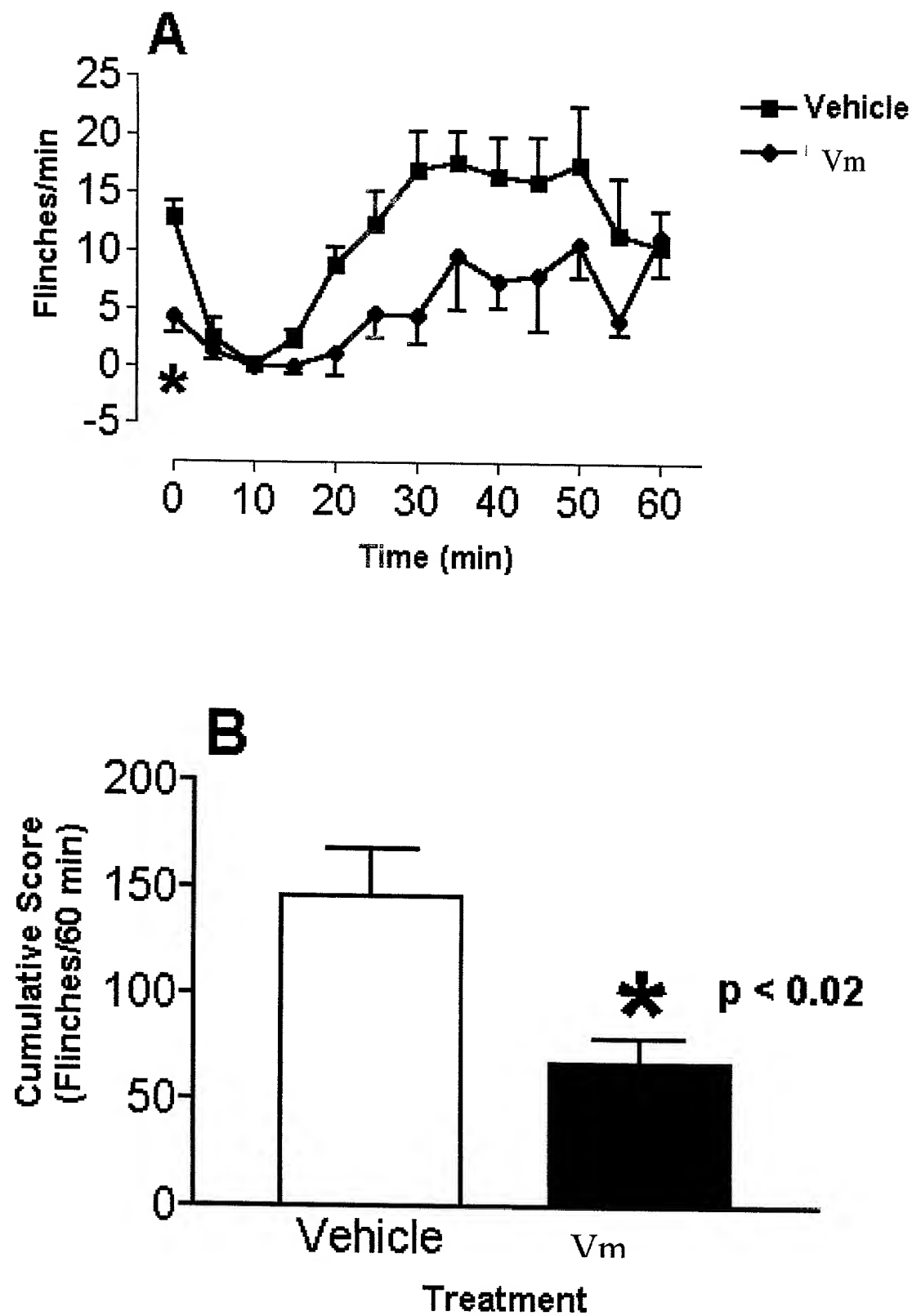
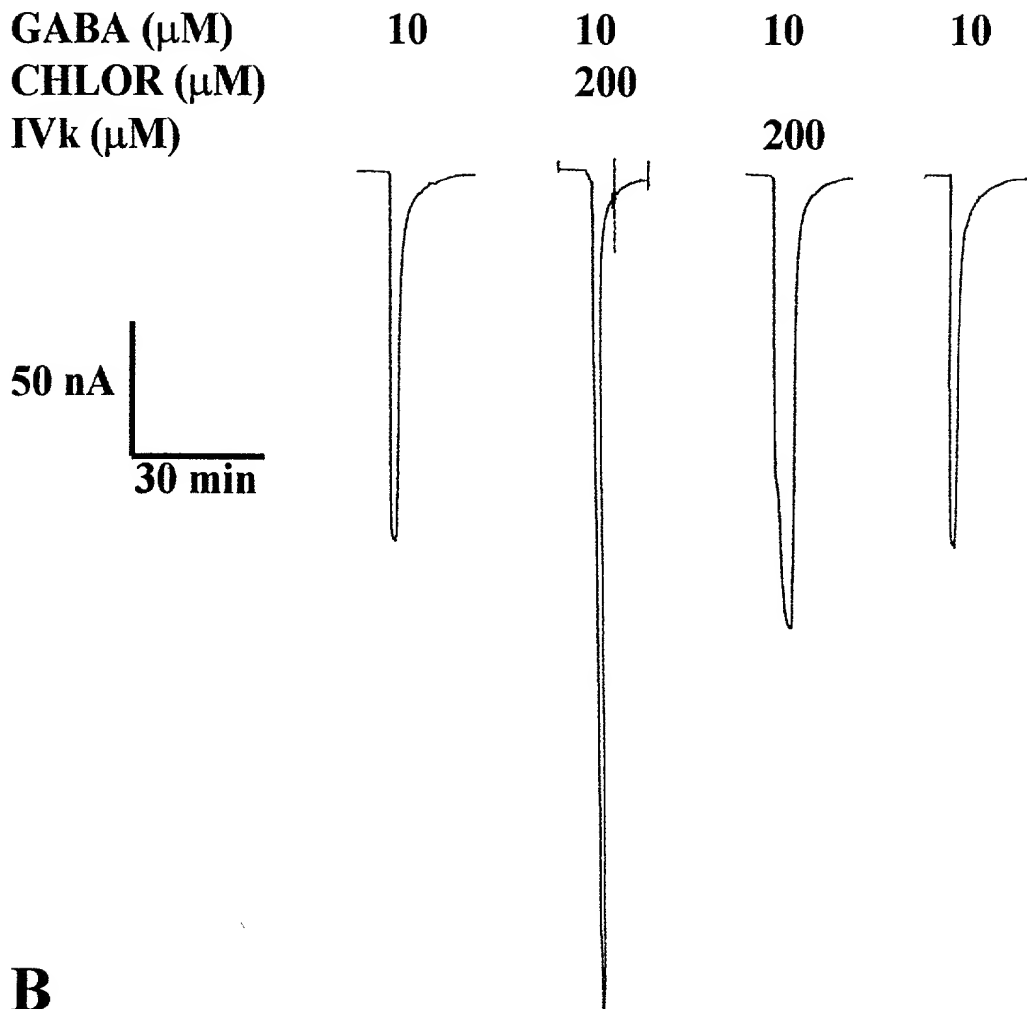


Figure 14



[illegible]

**GABA ( $\mu\text{M}$ )**  
**CHLOR ( $\mu\text{M}$ )**  
**IVk ( $\mu\text{M}$ )**



IVk (mg/kg)	n	Loss of Righting Reflex (min)
100	3	~15
200	3	~90

## DECLARATION

As the below named inventors, we hereby declare that:

Our residences, post office addresses and citizenships are as stated below next to our names.

We believe that we are the original, first and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled:

### **Methods and Compositions for Mitigating Pain**

the specification of which is attached hereto.

We hereby state that we have reviewed and understand the contents of the above-identified specification, including the claims.

We acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56.

We hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application

			Priority Claimed
			[ ] [X]
			Yes No
_____	_____	_____	
(Number)	(Country)	(Day/Month/Year Filed)	

## POWER OF ATTORNEY

We hereby appoint Carol Miernicki Steeg (Reg. No. 39,539) and Stephen J. Scribner (Reg. No. 44,452), each care of PARTEQ Innovations, Queen's University, Kingston, Ontario, K7L 3N6, Canada, as our attorneys or agents to prosecute this application, to make alterations and amendments therein, to receive the patent and all correspondence relating to this application, and to transact all business in the United States Patent and Trademark Office connected therewith, and the said attorneys or agents are hereby given full power of substitution and revocation.

Please address all correspondence concerning this application to Stephen J. Scribner, PARTEQ Innovations, Queen's University, Kingston, Ontario, K7L 3N6, CANADA.

We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these

statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of inventor: Gregory R.J. THATCHER

\_\_\_\_\_  
Inventor's Signature

\_\_\_\_\_  
United Kingdom  
Dated                      Citizenship

371 Alfred Street  
Kingston, Ontario, K7K 4H6, Canada  
Residence and Post Office Address

Full name of inventor: Brian M. BENNETT

\_\_\_\_\_  
Inventor's Signature

\_\_\_\_\_  
Canada  
Dated                      Citizenship

36 Fairway Hill Crescent  
Kingston, Ontario, K7K 2B4, Canada  
Residence and Post Office Address

Full name of inventor: James N. REYNOLDS

\_\_\_\_\_  
Inventor's Signature

\_\_\_\_\_  
Dated                      Canada  
   Citizenship

52 Herchmer Crescent  
Kingston, Ontario, K7M 2V9, Canada  
Residence and Post Office Address

Full name of inventor: Khem JHAMANDAS

\_\_\_\_\_  
Inventor's Signature

\_\_\_\_\_  
Dated                      Canada  
   Citizenship

17 Jorene Drive  
Kingston, Ontario, K7M 3X5, Canada  
Residence and Post Office Address